

Must 1992 be postponed?

The European Community, perpetually in crisis, is about to make a series of crucial decisions as well as to elect a parliament. It would be well advised to pay more attention to the second task than is its habit.

Is the European enterprise running off the rails? That must be the question prompted by the mounting list of quarrels between the twelve supposed partners in the European Economic Community (EEC). The most divisive row is about the plan that the creation of a genuinely common market by the end of 1992 should be accompanied by monetary union — a common central bank, a common currency and all that. Optimists hope that issue will be settled at a meeting of the heads of government in Madrid at the end of next month, but others expect it to persist. Meanwhile the British government, using tactics very much like those of the French government in the early 1960s, has taken to resisting proposals that it considers infringe pointlessly on national sovereignty. Last week, in Brussels, it was even fighting a plan that cigarette packets should carry the same health warnings everywhere in Europe. Now, to rub salt in fresh wounds, there are elections to the European Parliament in the next two weeks or so.

The trouble from which these quarrels stem is well-known, but is not openly acknowledged either by the European Commission or the twelve member governments. The EEC began life as a customs union, a deal between member governments to remove barriers to trade among themselves and to erect common barriers (tariffs, etc.) against the outside world. When, after more than a quarter of a century, the Treaty of Rome was plainly inadequate for the purpose, it was amended (by the Single Act of 1986) to limit members' freedom to veto proposals that did not suit them. But apart from high-sounding phrases, there is nothing in the treaties requiring member governments to act in concert on matters other than mutual trading policy and the consequences thereof except for the two general provisions of the Treaty of Rome that national legislation should in due course be "harmonized" (Article 100) and that EEC legislation might, by agreement, cover matters not originally included (Article 235).

The difficulties in the present situation are obvious. One is that the ramifications of trading policy touch every aspect of public affairs. (National health warnings on cigarette packets might be manipulated to keep Gauloises out of Britain, for example.) So long as the EEC lacks a constitution (such as that of the United States) that comprehensively regulates relations between the centre and its constituent parts, continual compromises will be

necessary between the zeal of those driving for a united Europe and the treaty rights of member governments. But that points to a second obvious snag: the EEC has no effective machinery for winning general political consent for those of its innovations that raise serious political issues for member governments.

But is that not the function of the European Parliament? Sadly, for much of the EEC, electing people to the parliament is a little like the practice on Noah's ark of releasing doves from time to time so as to tell whether dry land was within reach: each election seems to matter very little, although everybody expects that, one day, the elections will be crucial. The parliament should be, and eventually may be, an everyday arbiter of the conflicts that must inevitably arise when the European Commission claims within its administrative competence matters that seem to at least some member governments to be unreasonable intrusions of national sovereignty. But the parliament as it is is not nearly that judicious.

Take, for example, another of the issues disputed by the British government at Brussels last week, that of whether the European Commission has the right to regulate the quality of the public water supply throughout the EEC. The British government is within its rights to say that the European treaties do not remove its right and responsibility to strike an economic balance between British public health and public water quality in the light of how much protection British consumers are prepared to pay for, either in taxes or in water charges.

It might be possible to make an economic case for uniformity by saying that countries with poor-quality water would win an economic advantage thereby or even, if the quality of the water were notoriously bad, would hamper the free movement of workers within the EEC by frightening away the timorous. But those are trivial considerations, and the proposal that there should be uniformity is in any case sustained by the catch-all articles 100 and 235. A parliament with political horse-sense would be able, without prompting, to persuade everybody concerned, the Commission and member governments, to accord this issue the low priority it deserves amid the welter of decisions that must be made if 1992 is to happen.

Unfortunately, that is not what the European Parliament is like. Constructive compromise is not its strong suit. Instead, it seems bent on the advocacy of extreme



positions. That should be plain from its latest decision on the use of hormones in the livestock industry. Unabashed by the disgraceful decision in 1987 to ban, without evidence of harm, the use of anabolic steroids in livestock feed, and by the rumpus with the United States that followed, the parliament resolved on 14 April that the Commission should now go further and ban the administration to domestic animals of any substance not intended to be therapeutic (for the animals). To make sure, the parliament asked that only veterinarians should be allowed to administer artificial substances to animals, thereby casting doubt on the use of home remedies for the treatment of domestic pets. But the general tone of the resolution is hysterical: member states are urged to suppress black markets in hormones, the EEC to use its influence to extend the ban world-wide and the parliament comes out against the registration for use in animals of "new genetically engineered growth hormones".

European democrats will say that European voters can expect no better so long as they fail to take the election of the parliament seriously, but that is only half the story. Neither the Commission nor member governments of the EEC wish to see a stronger European Parliament, knowing as they do that their own influence would then diminish. The result is that most of the members of the European Parliament, of whatever political stripe, are more devoted to the concept of a united Europe than are their electors, but are otherwise prone to make grand causes of the bees buzzing in their bonnets. The European Parliament seems especially bankrupt of promise in the present circumstances of the great row building up over monetary arrangements.

The issues are neither as complicated nor as arcane as they are made to seem. What is meant to be a common market will not work efficiently unless those selling goods and services know in advance what they will get for them, which argues for the system of fixed exchange rates called the European Monetary System (EMS, to which Britain has so far chosen not to belong). But there is a case for going further than EMS. First, if all EEC members decide to let their citizens shift money where they choose within the EEC (one of the elementary provisions of the Treaty of Rome largely honoured in the breach, but Britain is an honourable exception), there is a possibility that members might compete for each other's capital with competitive interest rates, which argues for monetary uniformity, best secured by a European central bank. But having gone so far, why not go the whole hog, simplifying in the process, by creating a common currency unit allowing Europe's exporters (to each other) to be paid on the nail, without waiting days for their banks to decide how much is due to them?

Those are the three steps towards monetary union advocated last year by a group under M. Jacques Delors, the President of the European Commission. The logic is impeccable, but its application is disputed, at least by the British, who are nevertheless delicately divided in their opposition: Mrs Margaret Thatcher, the Prime Minister,

will have none of it, but Mr Nigel Lawson, the Chancellor of the Exchequer, set out nearly two years ago to pretend that Britain already belonged to EMS by instructing the Bank of England to rig interest rates so that sterling seemed to shadow the deutschmark. Lawson failed to predict (but might have guessed) that the coincidence of low interest rates with substantial cuts in income tax a year ago would stain his reputation as a money-manager. Thatcher, having accused him of mismanagement a week ago and then, with uncharacteristically diplomatic sensibility, having said that she was mistaken, made credible his statement at Madrid last weekend that Britain would at some stage join EMS, presumably on or about 31 December 1992.

Curiously, this is another tight corner in which an effective European Parliament could make European disagreements more seemly and even assist the grander European cause. The reason why politicians are generally reviled is that they are skilled at reconciling divergent opinions with their own (which makes them seem unprincipled). Their strength is that their sense of what issues are likely to stir electorates is usually more accurate than other people's. The trouble with Delors' proposals on European monetary union, as with the disagreements between Thatcher and Lawson, is that they rest on abstract arguments whose practical relevance to voters' lives seems remote, and which are generally not understood.

The trouble with the European Parliament is that there is no obvious way in which, even after the elections now due, its attention could be directed towards the constructive resolution of these conflicts. Yet a sensible parliament, particularly a parliament of Euro-zealots, would put first things first and recognize that it is more important to see that 1992 eventually happens than that there is all-embracing legislation on the control of how domesticated animals are cared for at the turn of the century, or whether water supplied from public sources is identical in Newcastle and Naples.

Many more immediate issues cry out for attention, not the least of which is EEC policy on research. With masterly insouciance, the European Commission, member governments and even candidates seeking election to the European Parliament have fallen in with the assumption that the Commission should spend the funds at its disposal on pre-competitive research and development (whatever that is) involving the collaboration of at least two national consortia. That the formula may be inappropriate after 1992 appears not to have been noticed. That European research spending should instead be concentrated on the improvement of the infrastructure — higher education and basic research — would most frequently be regarded as a defence of the interests of the effete. In an ideal world, in the impending elections, one would vote for whoever said as much. Sadly, the chance is very high that none of the candidates will even bother to pronounce on the issue one way or the other. But that is why 1992 may have to be postponed. □

Daunting costs for clean-up at Hanford

- Large amounts of waste involved
- Millions of dollars needed

Boston

OFFICIALS from the United States Energy Department and from Washington state signed an agreement last week that sets in motion a massive 30-year project to clean up Washington's Hanford Military Reservation, the nation's most troubled nuclear fuel production facility. The agreement outlines the legal and technical details of the clean-up, establishes a timetable for the project, and commits the Energy Department publicly to the effort. It does not, however, include an appropriation of money to do the job, which must come from Congress.

The Hanford agreement was welcomed in all quarters. In a written statement, Energy Department Secretary James D. Watkins declared Hanford's clean-up to be "of utmost importance" and added that the agreement "properly emphasizes" the attention that the job requires from the federal agency.

Washington Governor Booth Gardner hailed the fact that the clean-up would begin "at long last". And representatives from environmental groups and from the regional office of the Environmental Protection Agency say the agreement can be used as a model to guide the clean-up

efforts at other Energy Department facilities around the nation.

But all parties are concerned about the feasibility of the clean-up agreement because of the expense involved. The scale of the effort will be unprecedented. According to the Energy Department's own estimates, the project could cost as much as \$57,000 million, requiring a sustained federal outlay of close to \$2,000 million a year to get the job done on time.

Expenditure on that scale at a single site is almost inconceivable in the current fiscal environment according to several congressional insiders, especially because Hanford is only one (albeit the largest) of the clean-up projects that will be required at the Energy Department's 16 weapons production facilities. The Energy Department's entire budget for fiscal year 1989 is roughly \$14,000 million.

The enormous cost of the project stems in large part from the amount of waste involved. At the 560-square mile Hanford Reservation is an estimated 30 million cubic feet of nuclear waste and perhaps as much as one hundred times that amount of contaminated soil — the accumulation of more than four decades of radioactive by-products of plutonium production at

the facility. Workers at the Hanford site conducted the world's earliest large-scale effort to produce weapons-grade plutonium, and manufactured the plutonium used in the bomb dropped on Nagasaki in the Second World War. Following the war, Hanford served as a key location for military plutonium production and processing, housing a total of nine production reactors, all of which are now shut down.

The astronomical cost of the project also reflects the high level of radioactivity present in some of the waste and the difficulty of handling it in its current state. More than 500,000 gallons of high-level liquid wastes are known to have leaked from at least 58 underground tanks at the site, and much more leakage is suspected at another 100 tanks. The leached waste liquid and the remaining sediment in the tanks themselves — both extremely radioactive — present a daunting technical challenge for the environmental restoration project. The Energy Department has yet to offer specific technical solutions.

The high cost of the project also reflects the variety of clean-up activities required. In addition to the high-level liquid wastes found on the site, Hanford's nuclear reactors themselves contain radioactive residues and must be dismantled (see *Nature* 339, 90; 11 May 1989). Last month, the Energy Department issued a 300-page environmental impact statement showing the gigantic scale of just this part of the clean-up, with costs estimated at nearly \$200 million.

Environmentalists involved in the issue reacted favourably to the Hanford agreement but representatives from several groups expressed dismay over "lax provisions" in addressing current practices at the site. Lindy Cater, executive director of the Hanford Education Action League (HEAL), which is credited with publicly disclosing many of the environmental problems at the site, complains that while the agreement covers existing waste, it "fails to address ongoing waste production at Hanford's PUREX plutonium processing facility".

HEAL had urged previously that the clean-up agreement be tied to a stop to plutonium processing at PUREX, which according to one estimate produces 23 million gallons of water containing low levels of radioactive and chemical wastes in every day of operation.

In response to these complaints, the state of Washington announced that, in addition to the agreement, it will take part in a 14-month investigation of the current waste stream from Hanford's plutonium-processing facility. Officials said that this investigation will seek to determine the threat posed by the waste stream, and whether the state will call for a halt to processing before the 1995 deadline in the agreement.

Seth Shulman

ATMOSPHERIC SCIENCES

NERC plans to double its expenditure

London

THE UK Natural Environment Research Council (NERC) plans to double its expenditure on atmospheric sciences in universities next year, giving priority to global atmospheric modelling, tropospheric chemistry and meso-scale meteorology. And to highlight the new emphasis, the council is creating a new 5-year post for a director of atmospheric sciences; setting up a committee to distribute research funds in this area; and renaming the Marine Sciences Directorate as the Marine and Atmospheric Sciences Directorate.

John Bowman, secretary of NERC, says that until now the atmospheric sciences have been the 'Cinderella' of the NERC: research funds have been scarce and the research community is small. Last year, NERC spent only £1.5 million (1 per cent of the research budget) on the atmospheric sciences (excluding about £5 million on the British Antarctic Survey).

The council is requesting £1.5 million more from the national science budget next year. The sum is modest admits Bowman,

but any more would be disproportionate to the size of the research community. The council plans to increase resources gradually. In the first policy document for the atmospheric sciences, published last week, priority areas of research were outlined.

More than half the money requested for next year will be spent on UGAMP (the Universities Global Atmospheric Modelling Project) which develops climate models and analyses their products. It currently involves only 20 researchers and has a budget of only £100,000.

If NERC's request is approved, UGAMP's budget will increase to £800,000, an indication that the council is finally taking the atmospheric sciences seriously, says Professor Brian Hoskins of the University of Reading, a member of the UGAMP steering committee. If there were no increase in resources, the prospects for the research community would be "dire". The atmospheric sciences desperately need to attract good chemists, physicists and mathematicians, he says.

Christine McGourty

Bandwagon heads east

Tokyo

JAPAN'S Environment Agency, which has recently been talking tough (and getting its way) on everything from the protection of the ozone layer to the preservation of Okinawa's coral reefs, is calling on Japan to play a bigger role in protecting the global environment in two reports released last week.

The newly formed Global Environment Protection Bureau, made up of senior agency officials, urges the government and the private sector to contribute more to global environmental protection, particularly in developing countries of the neighbouring Asia-Pacific region. The report came a day after the release of the agency's annual white paper (see below) which also stresses the need for Japan to

participate actively in international efforts to protect the world's environment.

To promote more research, the bureau calls for monitoring of the ozone layer, rain-forest destruction, acid rain and marine pollution in Japan and the Asia-Pacific region using satellites, ships, aircraft and laser radar. It also recommends the establishment of inter-agency research involving different government ministries (not easy in Japan) and urges active participation in the UN Environment Program (UNEP), the Intergovernmental Panel on Climate Change (IPCC) and international research efforts such as the International Geosphere-Biosphere Program (IGBP).

To help developing countries, the report calls for increased overseas development aid for projects related to the environment and the design of energy-saving technologies and environmental monitoring and protection systems suited to their special needs. The agency also offers to draw up stricter environmental guidelines for Japanese private industries investing overseas.

Finally, the report urges the government to develop a 'global green strategy' to minimize tropical forest destruction, desertification and loss of biodiversity in the world. Among the measures called for are greater support of the International Tropical Timber Organization (ITTO) and its efforts to promote sustainable forest management, Japanese help in establishing national parks and preserves in developing countries and education to raise the Japanese public's consciousness of environmental issues, including the introduction of 'ecomark' labels for products that have little adverse effect on the environment. The report also says its time for Japan to "come to grips" with the need to protect wildlife in developing countries. Japan has been severely criticized for its role in the destruction of tropical rain-forests and for permitting trade in endangered wildlife.

Despite international censure, Japan has made important contributions to protection of the global environment, according to Hisakazu Kato of the Environment Agency. Japan reacted swiftly to the oil crisis of the 1970s and through efficient use of energy produces far less carbon dioxide per head from the burning of fossil fuels than does the United States.

International concern over the global environment has strengthened the Environment Agency's own political clout. The proposals in the agency's reports will most probably set Japanese policy at the summit meeting of the advanced nations in Paris later this year. The global environment will be a key issue.

David Swinbanks

JAPANESE ENVIRONMENT

Midsummer days and tropical nights in Tokyo

Tokyo

TOKYO residents are already sweltering under the effects of man-induced climate change, according to the Environment Agency's latest white paper released last week.

The white paper, subtitled "Toward Ecopolis — the city where man lives in harmony with the environment", shows that the number of "midsummer days" (days when the temperature exceeds 30 °C) and "tropical nights" (nights when the temperature fails to drop below 25 °C) in Tokyo is soaring. Tropical nights are ten times as frequent as a century ago.

The agency says summer temperatures are rising because of heat generated by cars, machines and electrical appliances and radiation reflected from buildings and roads. Ironically, the 'heat-island effect' is greatest at night during Tokyo's hot muggy summers when sunlight is strong and residents switch on their air conditioners.

Coolers should simply suck heat out of the room and pump it outside the home without adding heat. But even the best coolers are only about 30 per cent efficient, says Hisakazu Kato, a division director of the Environment Agency, and they give off a lot of excess heat.

Traditional wooden homes simply relied on summer breezes to keep occupants cool. But Tokyo is fast turning into a city of concrete apartments, each with its own air conditioning. The white paper calls for centralized district heating and cooling systems to help a return to an "ecologically oriented urban infrastructure".

David Swinbanks

Hubble telescope faces further delay

Washington

THE National Aeronautics and Space Administration (NASA) continues to shuffle its shuttle launch schedule, and for astronomers this means another delay — albeit a slight one — for the Hubble Space Telescope.

With Magellan on its way to Venus after a successful launch aboard the space shuttle Atlantis, NASA officials are upbeat about what promises to be an exciting year for space science.

But they are also being cautious, in order, to "protect" two planetary missions that have critical launch windows. Only one of two planned Department of Defense missions will now go ahead before the scheduled 12 October launch of the Galileo mission to Jupiter. The Hubble Space Telescope will be launched after just three intervening missions: two military missions, and a mission to deploy a communications satellite. The space telescope launch is now unlikely to occur before February 1990.

Other science missions scheduled for launch on the shuttle in the next 18 months or so include Astro 1, a series of onboard astrophysics experiments, the Gamma-Ray Observatory, a companion to the Hubble Space Telescope in the great observatories series, and Ulysses, the joint mission with the European Space Agency to explore the Sun's poles.

COBE, the Cosmic Background Explorer, originally scheduled for a June launch on a Delta expendable launch vehicle, has now been delayed until November.

Joseph Palca

GENOME MAPPING

Support ends at Yale for mapping library

Washington

THE Howard Hughes Medical Institute plans to end its support of the Human Gene Mapping Library at Yale University in August 1990 in the expectation that funds will be provided by the federal genome project. Since 1985, the institute has contributed \$1 million a year to the operation of databases at Yale containing the current human genetic map, restriction fragment length polymorphisms, genetic probes, lists of investigators studying human genetics, and literature citations on human genetic disease.

The institute has hired Peter Pearson from the University of Leiden in the Netherlands to devise a new generation relational database drawing upon the Yale library headed by Frank Ruddle, and another database on human genetics run by Victor McKusick of Johns Hopkins University.

Carol Ezzell

NUCLEAR FUSION

Lukewarm praise for effort

Washington

A MODEST increase in the US research programme on the creation of sustained thermonuclear fusion by magnetic confinement is recommended in a report issued last week by the National Academy of Sciences* but, in recognition of the present political mood, the report also calls for efforts at international cooperation and the involvement of domestic industry in building the next round of machines.

Magnetic confinement devices aim to create the conditions for a continuous thermonuclear 'burn' by holding a plasma of deuterium-tritium fuel in a cocoon of strong magnetic fields at high enough density and temperature for fusion reactions to begin. But although experimental machines of increasing sophistication have been built since the 1950s, a sustained fusion reaction has not been achieved, and the present generation of 'tokamaks', represented by the Joint European Torus (JET) at Culham Laboratory in England and similar devices at

the Princeton Plasma Physics Laboratory (PPPL) in the United States and in Japan and the Soviet Union, will not achieve that goal.

Although all the countries with fusion programmes are pondering the next step, the prospects for international collaboration are complicated by disagreement over what experimental goal to attack. In the United States, plans are in hand for the Compact Ignition Tokamak (CIT), whose purpose is to persuade the hot plasma to ignite but not necessarily to create a sustained burn, whereas in Europe the Next European Torus (NET) is being contemplated as a machine which will hold a hotter, denser plasma for longer than JET, but in which actual ignition may still not occur. The next step for the Japanese programme is likely to be something similar to NET.

The total cost of any single fusion machine runs to hundreds of millions of dollars, and none of the contemplated next generation of machines has been approved for construction. CIT has been given \$3.5 million for continued research, but the US Congress, in its presently parsimonious mood, shows no signs of releasing enough money to start building.

HYDROELECTRIC DAM

Two-month moratorium

London

THE Hungarian government has declared a two-month moratorium on construction of the controversial Nagymaros hydroelectric dam, pending the report of yet another official commission of inquiry. Public opposition to the dam on environmental and social grounds has been increasing over the past two years, but until now the Hungarian government has maintained that, although in hindsight the scheme has to be considered a mistake, it would now cost too much to cancel it. Furthermore, the Nagymaros dam was designed to function in conjunction with one upstream at Gabčíkovo in Czechoslovakia, and to withdraw unilaterally would constitute a breach of international contract. This latter point was stressed by Hungary's foreign minister Peter Varkonyi, a few weeks ago in a lecture at the Royal Institute of International Affairs in London. Soon after, however, Varkonyi was replaced as foreign minister by Gyula Horn, who has made it clear that he considers the agreement to halt construction the correct one.

The Czechoslovak government considers its part of the scheme, the Gabčíkovo dam, to be vital to its future energy plans. Construction work at Gabčíkovo is now virtually complete, but without the Nagymaros barrage to act as a trap, the Gabčíkovo station will never be able to

operate at full strength. Last week, Foreign Ministry press secretary Ivan Kulhanek said that the agreement on the construction and operation of the dams is binding on both sides. The ecological considerations now being urged by the Hungarians, he said, had been studied in depth long before construction started and the Hungarian moratorium had been declared for "political" reasons.

The Czechoslovak party paper *Rude Pravo* was even more explicit. The Hungarian government, it said, had been pressurized by opposition groups, who had "manipulated" public opinion.

Hungary could also face difficulties with Austria if the Nagymaros dam is cancelled. At the end of 1984, the Austrian Greens forced the cancellation of plans for a hydroelectric station at Hainburg, and the Austrian government then undertook to bear a considerable part of the construction costs of Nagymaros, with eventual repayment in the form of electricity. If the dam is not completed, the Hungarians will have to reimburse the Austrians — as well as making good the environmental damage. The anti-dam campaigners have argued for years that cancellation would be cheap at the price. For the first time, there are indications that the Hungarian government may be beginning to share their view.

David Lindley

*Pacing the magnetic fusion program. National Academy Press, 1989.

GENETIC MEDICINE

Go-ahead for gene transfer experiment

Washington

AN experiment to insert foreign genes into humans started this week, following the settlement of a lawsuit brought by Jeremy Rifkin of the Foundation on Economic Trends. Under the terms of last week's settlement, the National Institutes of Health will alter the charter of the Recombinant DNA Advisory Committee (RAC) to make the approval process for gene transfer protocols more open and explicit.

W. French Anderson of the National Heart, Lung and Blood Institute and R. Michael Blaese and Steven A. Rosenberg of the National Cancer Institute proposed their experiment last June. A special class of activated lymphocytes with antitumour properties will be marked with a bacterial gene and then reinjected into human cancer patients.

Last October, RAC approved the experiment, but did so without waiting for a decision about its appropriateness from a subcommittee specially formed to judge the scientific and ethical questions raised by the protocol. The altered charter requires that a subcommittee must vote on all proposals referred to it before they can be taken up by the parent committee.

If the subcommittee ignores the proposal, investigators can appeal their case to the full committee. And all votes must now be taken in public unless they may be legally held behind closed doors. Joe Palca

Is France going green?

Paris

FOUR years after French government agents sank the Greenpeace ship *Rainbow Warrior*, the environmental action group is to try again to persuade the French public to back its international campaigns. Greenpeace has opened a new office in Paris with a complete change of staff and a 'softly softly' approach to win back the 20,000 former members who became disaffected after the 1985 affair, which resulted in the death of a Greenpeace photographer.

Meanwhile, the imminent election of delegates to the European Parliament, according to opinion poll-watchers, could see between 10 and 15 per cent of votes go to the new ecology party, mostly at the expense of the political right wing.

The *Rainbow Warrior* affair was not the only reason for the demise of Greenpeace in France, although subscriptions plummeted as a result and the group came to be perceived as anti-French. Between 1985 and 1988, internal squabbles caused a split in the staff, and financial mismanagement left the office with debts of FF2 million (\$306,000). But in January last year, Greenpeace International, which directs the activities of the 20 national agencies, decided to wind up the Paris office and to create another, Greenpeace-France, with a new director and staff. The FF50-million damages paid to Greenpeace International by the French government have meanwhile been used to build a new *Rainbow Warrior*, due to be launched in Hamburg in July.

The future of Greenpeace-France could prove to be a better barometer of French national opinion than the recent success of the ecology party in municipal elections. The political centre is extremely volatile, as was shown in last year's general elections when the balance of power shifted from the extreme right to the extreme left between the two rounds of voting. But the organization's new director, Philippe Lequenne, has no illusions about the difficulty of his task. France has recently clashed again with Greenpeace over the construction of a landing strip in the Antarctic, and last month the prime minister said that France would not ratify a new convention supplementing the Antarctic Treaty (*Nature* 339, 8; 4 May 1989).

A paradox is that support remains for nuclear energy. "French people love technology but hate change", says Lequenne. To make matters more difficult, the former director of Greenpeace in France claims that the company was wound up illegally and is considering taking legal action.

Peter Coles

Money promised for science

London

THE main opposition party to Mrs Margaret Thatcher's Conservative government in Britain, the Labour party, promises increased investment in civil research and in higher education if it gains power at the next general election, due in 1992.

In a policy document published last week after a two-year review, science takes high priority. Labour plans the creation of a more scientifically literate population and the construction of a solid basis for future industrial success.

The creation of the post in government of minister for science and technology within the Department of Trade and Industry is central to a new science programme. This minister would be responsible for the distribution of the extra resources for civil research to help science recover from the underfunding of recent years, says Labour. No figures for investment are given; the sum needed is small, it says, but is essential if Britain is not to "run out of both scientists and ideas".

The increasing trend towards concentration of research specialisms in individual universities is welcomed as "inevitable and economically desirable". Centres of excellence should be encouraged, says Labour, but not to the extent that some smaller universities end up doing no research at all.

A new climate in higher education would replace the "turmoil, cuts and demoralization of the Conservative years", to the benefit of all staff, says Labour. Researchers would be offered "good career prospects", and tenure, which was abolished by the government last year, would be reintroduced but made dependent on merit.

In agreement with the present government, Labour would seek to increase significantly the numbers entering higher education, providing financial incentives for institutions to attract not just greater numbers, but more students from a wider range of backgrounds, including women, mature students, those of ethnic origin and those with non-traditional qualifications.

Strongly opposed to the government's planned scheme to replace the maintenance grant with loans, Labour argues that the plan is expensive and limits access and student course choices. Instead, Labour would retain the grant system, review parental contribution and ensure that those who are married or over 21 are treated as independent for grant purposes.

A wide range of new measures would be introduced to encourage industry to increase investment in research. An investment institution, to be called the British Technology Enterprise (BTE), is proposed. Designed to overcome the ob-

session of the City of London with short-term profits, the new institutions would accept a lower rate of return over a longer payback period. The new BTE would resemble the successful National Enterprise Board set up by the last Labour government which produced Celltech, the biotechnology company, and INMOS, the electronics company famous for creation of the transputer. BTE might focus initially on opto-electronics, biotechnology and new materials.

Other plans to encourage industrial investment in research include the simplification of existing government support mechanisms, which Labour says are confusing, *ad hoc* arrangements. It would also use the tax system to encourage greater expenditure on research and to penalize those who spend too little. And to encourage technology transfer between higher education institutions and industry, a regional network of "technology innovation centres" would be set up.

Environmental policy has high priority in Labour's new policy document. The present Conservative government is criticized for focusing on "short-term, single-issue" research projects, revealing "a profound misunderstanding of what scientists do and what scientific knowledge is". Labour promises to support long-term programmes of environmental monitoring and to support basic research with the aim of anticipating environmental problems before they become urgent. Among the priority research areas would be the study of biological processes in the oceans, geophysical processes in the atmosphere and genetically engineered organisms in the environment. A joint research council should be set up to coordinate support for research on the global environment in Britain, and a European environmental charter should be drawn up to encourage cooperative research in Europe. Labour also acknowledges a "compelling obligation to provide the developing countries with environmentally benign technologies."

Labour has toned down its objections to nuclear power, acknowledging that in the medium-term it will remain in use. But it labels the government's promotion of nuclear power in the face of the greenhouse effect "a transparent piece of opportunism", and pledges instead to focus on energy conservation and the development of alternative sources of energy.

It also promises public investment in a "new electronic highway" for Britain, through the construction of a national broad-band fibre-optic cable network. In contrast to the Conservative government, Labour says that as this communications structure would promote economic growth, public investment is necessary.

Christine McGourty

JAPANESE SPACE POLICY

Hopes for planetary boost

Tokyo

A MAJOR shift in Japan's space policy may soon free the Institute of Space and Astronautical Science (ISAS) from limits on the size of its launch vehicles, enabling it to begin laying ambitious plans to send Japanese space probes to the planets. The change in policy is proposed in a 10-year plan submitted by the long-term policy committee of the Space Activities Commission last week. The commission is expected to reach a final decision on the proposal by the middle of June.

The limit on ISAS's rockets was fixed 23 years ago when the National Space Development Agency (NASDA) was being set up to manage Japan's commercial space programme. ISAS (then part of Tokyo University) was allowed to maintain its independent, academic space research programme on condition that its home-developed solid-fuel rockets did not get too big — a limit of 1.41 metres tail diameter was set.

ISAS engineers have shown great ingenuity in working within the limit. In 1986 they sent two probes to Halley's comet and next year they plan to launch a probe to the Moon. But beyond the lunar probe, little more can be done with a small rocket.

For the past two years, the long-term policy committee has been considering proposals for a bigger rocket for ISAS, an

unmanned shuttle to be built by NASDA and Japanese manned space flight. A bigger rocket for ISAS has been a contentious issue because some would like to see NASDA's H-2 liquid fuel rocket, which is currently under development, used for planetary missions.

In the 10-year policy submitted to the commission last week, it is stated that ISAS should be allowed to build rockets that conform to the safety limits of the institute's Kagoshima space centre.

Although no specific size of rocket is mentioned, this will in effect allow ISAS to build a rocket that breaches the 1.41-metre limit. The committee's decision was made on the grounds that this is the most efficient and economical way to make use of Japan's rocket launch capability.

The rocket envisaged by ISAS will have a tail diameter of 2.5 metres, will be three times more powerful than the institute's present largest rocket, the Mu 3S-II, and will be able to place a 2-ton payload in low Earth orbit, slightly less than NASDA's present H-1 rocket. Development costs are expected to be about ¥15,000–20,000 million (\$108–\$144 million) with a price for each rocket of ¥5,000–5,500 million — cheap by anybody's standards but it will require a significant boost in ISAS's annual budget from the Ministry of Education, Science and Culture (MESC).

If the Space Activities Commission

gives approval and MESC provides the funds, the rocket could be ready for test launch in 1994, according to Jun Nishimura, director-general of ISAS. Among the missions being considered for the new rocket are a lunar probe that would drop penetrators containing seismometers into the lunar surface to measure 'moonquakes', a probe to Venus that could release a balloon into the Venusian atmosphere, and a mission to collect dust from a comet and bring it back to Earth for analysis. **David Swinbanks**

AMAZON FORESTS

Dispute enters new round

São Paulo

CONTROVERSY over the extent of deforestation in Brazil's Amazon refuses to go away. Last week, the Brazilian Society for the Progress of Science (SPBC, Sociedade Brasileira para o Progresso da Ciência) stepped into the arena by setting up a panel of three scientists to sort out the continuing claims, counterclaims, retractions and accusations flowing from figures for rain-forest destruction announced by Brazilian President José Sarney (see *Nature* 338, 531; 13 April 1989).

Sarney's figures, based on data from the Institute for Space Research (INPE), showed just 5.12 per cent of legal Amazonia had been destroyed; a very different picture from the 12 per cent estimate produced in a recent report from the World Bank. INPE was quickly criticized for erroneously relating the area destroyed to the total area of the legal Amazon, rather than to the area of rain forests, which is smaller. But INPE's director, Márcio Nogueira Barbosa, then accused the World Bank report of making the same mistake.

Other members of INPE's staff attacked the haste with which the initial report was produced for the president's announcement, forcing them to use old Landsat pictures in some cases (see *Nature* 339, 86; 11 May 1989).

In a debate in São Paulo last week, Barbosa announced that the team that produced the data for the president's announcement may write a more detailed and technical account of the work. He also said that all the satellite images used are available to the scientific community to look at.

During the same debate, the head of the SBPC committee that is studying the issue, the geographer Aziz Ab'Saber, of the University of São Paulo, said that the scientific community is worried about the damage to the Space Institute's image and that they want to discuss the methodology used in the assessment of deforestation.

Ricardo Bonalume Neto

EUROPEAN SCIENCE

EUREKA train drives collaboration

Paris

ON the initiative of the French ministry for research and technology, an 'exhibition train', featuring Europe's EUREKA programme for cross-frontier industrial collaboration in technology research, is set to wind its way through France, West Germany, Italy and Spain.

The initiative is both a celebration of the success of EUREKA — the brainchild of the French research minister, Hubert Curien — and an effort to solicit new proposals from regional industries.

The French government and industry have contributed almost 40 per cent of EUREKA's FF26,000 million (\$4,000 million) total cost since it was set up in 1985.

The 13-day train journey, with eight stop-overs for seminars, will end in Lyons on 19 June to coincide with a conference on 'EUREKA in the regions'. On the same day, European research ministers attending EUREKA's ministerial meeting in Vienna will announce a new batch of projects. Out of more than 100 proposals already received, over 60 are expected to be approved.

Although not officially on the Vienna

agenda, the new JESSI semiconductor programme of the European Commission (EC), which has not yet received full budget approval, will undoubtedly be dis-



cussed. It is intended that JESSI link up with EUREKA and the EC information technology programme, ESPRIT, but no decision has so far been reached. Reluctant to be pinned down, Curien said that the French government will "do its best to see that JESSI gets the money it needs".

Peter Coles

More to come but who pays?

Washington

THE National Science Foundation (NSF) last week renewed its commitment to four of five existing supercomputer centres by extending the cooperative agreements with them through the 1995 fiscal year. But the next six years will be crucial ones for the centres, as they gradually end their role as prototype facilities for high-performance computing and become part of the US scientific infrastructure.

Supercomputer centres, and enhanced access to them via new, high-speed networks such as NSFNet, have changed the profile of computational science in the United States. NSF's commitment to supercomputer centres began in 1985 fiscal year. The four centres whose grants were renewed are at the University of Illinois at Urbana-Champaign, the University of California at San Diego, the University of Pittsburgh-Carnegie Mellon University and Cornell University.

Approval would, in all likelihood, have also come to the John von Neumann Center near Princeton, New Jersey, but last month Control Data Corporation announced it was discontinuing its ETA line of supercomputer. The Princeton centre uses ETA computers, and its future expansion plans included the next generation of these, so the centre is now scrambling to find alternatives. A decision on renewal has been deferred until later this year.

Although it is NSF's intention to increase funding levels from an average of \$10 million per centre to \$14 million, any increase will be contingent on how Congress deals with the Bush administration's NSF budget proposal.

While the centre's directors anxiously await the news about how much they will receive in next year's budget, of greater concern to them is a proposal intended to start the following year that would alter dramatically the way the researchers are allocated time at the centres.

Thomas Weber, director of the division of advanced scientific computing at NSF, says the agency is thinking about instituting a "green stamp" scheme for time on supercomputers at NSF centres.

Under the plan, programme directors would be given an allocation of green stamps for grantees. These stamps could be redeemed to the centres for computing time.

Weber says this plan will give NSF control over how supercomputer time is allocated, and make certain that NSF programme directors know what their grantees are doing. He adds that this will eliminate one stage of the review process, as at present researchers must compete for time at the centres.

But some worry that the real impact of

the plan will be to drive people away from the centres. Doyle Knight, director of the von Neumann centre, says the green-stamp plan could lead to the same problem that struck university computing centres in the past decade. Knight says that after establishing the centres in the 1960s, NSF reduced support, leaving universities to find independent support. For many centres this meant charging users real dollars for computing. But users, faced with a new expense and the old problem of sharing a central resource, started buying mini-computers that were less powerful but affordable and did not have to be shared.

Knight says this caused two problems. On the one hand, support for large powerful computers declined, and on the other computational scientists were forced to spend a significant portion of their time as computer operators. Larry Smarr, director of the University of Illinois centre, says it was as if astronomers traded a large optical telescope for one thousand pairs of binoculars. Although they might have the same total light-gathering capacity, says Smarr, the science is limited. NSF's de-

cision to reduce support for university computer centres led to a supercomputer famine according to Smarr.

One goal of the green stamp programme — to spread support for supercomputing activities among the many NSF directorates whose grantees use supercomputing facilities — is a good one, says Smarr, but the green-stamp mechanism is the wrong one. "The stupid thing about green stamps is we have solved the allocation problem, not by money but by peer review." Each of the centres now has a peer-review committee to judge requests for what is now essentially free time on the computers.

Thomas Day, president of San Diego State University and vice-chairman of the National Science Board that approves NSF's plans, says the supercomputer issue is a crucial one for the country's science infrastructure. The centres reflect the paradox of NSF's role in US science — on the one hand to generate new ideas and new directions for science, and on the other to support existing programmes. Whether the board supports the green-stamp scheme, the tug from these two competing drives will require some form of compromise.

Joseph Palca

ENGINEERING RESEARCH

NEL privatization delayed

London

THE British government-owned National Engineering Laboratory (NEL) will be privatized several years from now, not within a few months as originally planned. After considering a report on the future of NEL from management consultants Touche Ross, the government announced last week that the laboratory would be allowed five to seven years in which to become profitable.

The chief scientist at the Department of Trade and Industry, Ron Coleman, told staff at NEL that when the necessary legislation is in place the laboratory will be transferred from the civil service to the ownership of a limited company, called the National Technology Centre. Initially, all the shares will be held by the government.

Over several years, the laboratory would be expected to undergo some restructuring in order to function as a commercial enterprise, including losing 200 of the 600 staff. To make better use of the space available at the NEL site, a science and technology park should be created around it.

The NEL, a mechanical-engineering laboratory, has supported industry by the transfer of technology at low cost. The government now insists that industry should pay the full costs of the research and development. But staff fear that the

laboratory may not survive in the private sector.

A representative of the Institute of Professional Civil Servants at the NEL, Stephen Marshall, says that research alone cannot be profitable, and the few existing independent research organizations may have saturated the market. Transferring the NEL into the private sector will be "a momentous task" and there are no assurances that the government will provide any support if it fails, he says.

The advice of management consultants was requested last year after the first hurried attempt at privatization failed. Announcing the sale, the government called for bids for the NEL, valued at about £30 million, within six weeks; negotiations with a potential buyer were unsuccessful.

Privatization has been ruled out for the moment for the government's environmental research laboratory at Warren Spring. It is to be given the status of an independent 'agency', which means loosening its ties to the DTI. The laboratory is expected to increase its income from private contractors and to compete for research contracts with government. The laboratory employs 300 staff and costs £10 million a year, more than two-thirds coming from government at present.

Christine McGourty

EUROPEAN COMPUTER NETWORKS

New network raises anxiety

Munich

PROVIDERS of European academic computer networks glimpsed the future in Europe last week and were not happy with what they saw.

A fledgling network named IXI (International X.25 Infrastructure), sponsored by the Eureka project COSINE, faces significant obstacles before it can become a worthy successor or partner to existing networks such as EARN and EUNET.

The scepticism of EARN and EUNET managers toward IXI, unveiled at the RARE 'Networkshop' in Trieste, Italy, on 9 May, was tempered with the knowledge that they will probably have to learn to live with the new network.

Powerful forces brought about the creation of IXI. The backers of the network include the association of research networks known as RARE (Réseaux Associés pour la Recherche Européenne)* and the Eureka project COSINE (Cooperation for Open Systems Interconnection Networking in Europe). RARE represents national networks such as DFN in West Germany, JANET in Great Britain and REUNIRE in France.

GERMAN COMPUTER NETWORK

Lower prices wanted

Munich

LESS expensive services for users are at the top of a list of requests submitted by the West German computer research network organization DFN (Deutsche Forschungszentrum) to the West German postal ministry. The ministry has already agreed to provide a network for researchers at transmission speeds up to 64,000 bits per second (b.p.s). A network for speeds up to 2 million b.p.s should be available within two to three years. Negotiations are still going on regarding a high-speed network for speeds up to 140 million b.p.s.

Telecommunications services will be deregulated in West Germany from 1 July, and Martin Wilhelm of DFN says that DFN has already looked at a relatively expensive offer from a private network.

But DFN, convinced that the postal ministry will offer better quality, has chosen to wait for the ministry to install and test its own 2 million b.p.s. network.

Other items on the request list include lower tariffs for local-area networks and continued good-faith participation in international negotiations to establish a European X.25 network for researchers (see above). DFN points out that researchers and the ministry have a common interest in the development of new data services and equipment. But whether the ministry will accede to the demands "will only come out in time", said Wilhelm. Steven Dickman

RARE and COSINE are negotiating with the PTT-Telecom of the Netherlands to install IXI in 19 European countries. Negotiations may be completed within the month. IXI will have a four-month start-up phase followed by one year of operation.

After that trial period, RARE and COSINE directors and the national networks they represent hope to know whether it will be feasible to use IXI as a basis for future academic networks.

The new network will eventually offer an X.25 backbone linking the data networks of as many as 19 European countries. The initial operating costs, estimated at 5 to 6 million ECU (1 ECU = \$1.09), will be shared by the member states in proportion to their gross domestic products and by the Commission of the European Communities. Eventually, IXI will provide a "harmonized X.400 service" for electronic mail and a pilot service for file transfer, as well as a gateway to North American research networks.

The main criticism voiced by managers of EARN (European Academic Research Network) and EUNET (European Unix Network) against IXI is that it costs too much. Dennis Jennings of Ireland, formerly president of EARN and now a board member, points out that EARN operates for about 2 million ECU a year. He is afraid that IXI will not be "cost-effective".

The chairman of the COSINE policy group, Peter Tindemans of the Netherlands, disagrees. He points out that many of the costs of operating EARN are hidden, borne by corporate subsidies or national network organizations. The real costs of EARN, he says — especially considering the initial investment in it made by IBM — are much higher. In the long run, says Tindemans, "ours will be a cheaper set-up".

EARN user Brian Carpenter, head of the communications group at CERN in Geneva, supports Tindemans. "It might be wise to pay more [for IXI] in order to raise the standards." EARN is frequently congested and cannot always provide the quality of service expected, he says.

A second worry is that IXI is supported by postal authorities (called PTTs) in each country. Although even Jennings agrees that academic networks need the cooperation of PTTs, which provide the leased telephone lines over which data is transmitted, he is worried that they will play too strong a role in operating IXI.

Because most PTTs are monopolies, researchers wonder if PTTs will take advantage of them.

A related fear concerns possible exploitation of IXI users by national network organizations such as REUNIRE and DFN. EARN has been mostly run by volunteers, whereas the national net-

SOVIET SUBMARINE

Search off Norway for Komsomolets

London

FOUR Soviet ships — the research vessel *Akademik Mystislav Keldysh* belonging to the Soviet Academy of Sciences, the hydrographic survey ship *Persei* and the rescue vessels *Georgii Titov* and *Mikhail Rudnitskii* — are trying to locate the Soviet submarine *Komsomolets* which sank in the Norwegian Sea on 7 April with the loss of 42 lives. According to the Red Army newspaper, *Krasnaya Zvezda*, the manned seabed exploration craft carried by the *Keldysh* will be used to photograph the sunken vessel. It stated emphatically that neither the Soviet Union nor the United States has the technology to raise a wrecked vessel from a depth of 1.5 km.

The loss of the *Komsomolets* has drawn attention to Soviet deficiencies in air-sea rescue techniques. *Pravda* has suggested that some of the money saved by Mr Gorbachev's promised defence cuts should be spent on improving the situation. And one of the new people's deputies, Gavriil Popov, who was elected by the Union of Scientific and Engineering Societies, has made it clear that in his opinion high-level bungling and cost-cutting on safety made the death-toll higher than it would otherwise have been. Vera Rich

works are professionally managed. Although IXI would certainly still need volunteers, especially in operating electronic mail, databases and other high-level services, many such services would be sold to users by IXI or its member networks.

Intensifying this fear is the suspicion that the interests of European computer equipment suppliers are being placed ahead of those of researchers. EARN was widely perceived as a "plot to sell IBM machines" from the United States to European users, says one network user. IXI, users fear, may allow a backlash, to the detriment of researchers. Daniel Karrenberg, network manager of EUNET in Amsterdam, says that the goals of IXI have "disproportionately more to do with European corporations and PTT needs than with user needs".

Tindemans requests patience from network users during the pilot phase of IXI. Eventually, he foresees a "simple situation" in Europe, where COSINE provides the infrastructure for basic network services and networks such as EARN operate at the national level.

EARN and EUNET have announced that they will take part in the pilot phase of IXI. Users wishing to influence the development of future networks are urged by COSINE officials to "make their requirements known" to the RARE secretariat.

Steven Dickman

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Student demand in Australia

SIR—Tania Ewing (*Nature* 338, 191; 1989) makes the point of decreasing student demand in Australia for places in science courses and of disenchantment with career prospects for non-tenured post-doctoral scientists. Both observations are accurate, but the data used to support the former proposition are presented somewhat ambiguously.

Entry scores (out of 500) of 320 (1987) and 300 (1989) were quoted for the faculty of science of the University of Sydney while scores of 295 (1988) and 279 (1989) were given for the faculty of science of the University of Melbourne. It should be noted that the latter scores are out of a usual maximum of 410. Furthermore, the faculty of science at the University of Melbourne has deliberately increased its student intake this year by approximately 10 per cent, and the lower cut-off score reflects this planned expansion.

In the United Kingdom (*Nature* 334, 393–394; 1988) and in Australia, science courses are progressively providing a broad framework for a wide range of career directions. It is this message that we are attempting to market to potential science students; unqualified interpretation of data will be counterproductive to such efforts.

T. W. HEALY
(Dean)

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Animal experiments

SIR—We wonder how many of your readers were concerned over a report published earlier this year which in our view caused an unacceptable level of suffering in the experimental animals concerned.

The paper in question was "Maturation and connectivity of the visual cortex in monkey is altered by prenatal removal of retinal input" (*Nature* 337, 265–267; 1989). The study involved major surgery on two pregnant macaques (*Macaca cynomolgus*) to expose the fetal heads; the eyes of both fetuses removed; six weeks to two months later the blinded animals were prematurely delivered by caesarean section. The infants were bottle-fed and, although it was not stated, presumably removed from their mothers. They were subsequently subjected to craniotomy for tests on the visual cortical area and were then killed.

This fundamental research deprived

two primates of their young and their young suffered the effects of maternal deprivation and loss of sight. Such gross interference with a highly intelligent species requires overwhelming justification — if it can ever be justified.

This work was performed in France and was therefore not subject to British law. We believe that such work would never be permitted in Great Britain under the Animals (Scientific Procedures) Act 1986. We therefore question the decision of *Nature* to permit publication of this work, even allowing for *Nature's* stated policy of publishing "what will be of the most scientific interest to our readers".

To my knowledge, no other reputable scientific journal published in this country will accept any reports of work which clearly would not have been authorized under British law.

CLIVE HOLLANDS

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Conference participation

SIR—All scientists should have an equal right to participate in international scientific conferences but unfortunately this is not the case, for several reasons.

There are two categories of countries — those with convertible currencies and those without them. Conference fees are rising more than inflation and they invariably have to be paid in US dollars or other convertible currencies.

This practice discriminates strongly against scientists from countries with no convertible currencies, and that is the larger and poorer part of the world. Western organizers of conferences forget too easily that for many of us — even when local funds are available — it is difficult if not impossible to obtain them in convertible form. They also forget that delivery of letters may take weeks rather than days in those countries and that the transfer of \$10 may be as hard a task as the transfer of \$10,000. I regularly receive conference circulars with requests for advance payments and with deadlines that are impossible to meet.

As a Hungarian scientist, I am under stress and feel miserable at being prevented from participating in important international conferences because of the convertible currency problem, and I am sure I am not alone in this respect. This situation will further widen the gap between the scientific achievements of developed and "not so developed" countries, which is in nobody's interest. Representatives of international scientific organizations such as COSPAR, IAU and

IUGG should seek a solution to this problem.

I should like to make the following suggestions to conference organizers:

- (1) They should accept participation fees in local (non-convertible) currencies and use them on other occasions within that particular country. National scientific committees or representatives could take care of the account.
- (2) They should set aside part of conference income to support the participation of scientists from countries without convertible currencies.
- (3) If the conference is organized in such a country, the local organizers should be obliged to support scientists with convertible currency at other conferences.
- (4) They should not be rigid about closing dates for advance payments and deposits but should deal with each case individually.

Such arrangements would make organizing a conference more complicated but they would allow a more balanced participation of scientists from East and West.

ISTVÁN FEJES

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A cost of progress

SIR—The evils properly associated with eugenics have caused us to forget that it did have a partly rational basis. Kondrashov¹ and Grinde² have remarked on the progressive increase in deleterious alleles to be expected as a result of advances in medicine and public health. This expectation has a distinguished history; Muller's classic paper³ putting forward the concept of genetic load has a mature (if not a comprehensive) discussion.

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2. Grinde, B. *Nature* 338, 24 (1989).
3. Muller, H.J. *Am. J. hum. Genet.* 2, 111–176 (1950).

Attendant problem

SIR—In his delightful review of the second edition of the *Oxford English Dictionary* (*Nature* 338, 385; 1989), Stephen Jay Gould discusses the word 'scientist'. He writes of 'attendees' at the meeting of the British Association in 1834 at which the word was first suggested. Has he not confused active and passive? Doesn't he mean 'attenders'? If 'attende' exists, it presumably means someone attended, for example a monarch or a bride, not someone attending.

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Mental illness — who cares?

Malcolm P.I. Weller

Public policies on psychiatric disability have increasingly centred on community care as an alternative to hospitalization. In the process, those most in need are being neglected.

THE crusading enthusiasm of recent decades for bold initiatives in psychiatric health care, stridently evident in Italy, the United States, Britain and now Portugal, has been fuelled by an unwarranted assumption that psychiatric hospitals are self-evidently undesirable, an assumption often accompanied by an equally uncritical advocacy of 'community care'.

But community care is a nebulous term. In the words of the Richmond Fellowship, it is "a slogan that diverts attention from the real issues of quality of life for the mentally disordered, distressed or disabled people". And it may mean different things to different people. Critics of the existing system fail to distinguish distress from illness while denying the reality of the latter.

The advocates of the new order and those with direct involvement in hospital-based care are engaged in a kind of trench warfare, while the psychiatric hospitals, already grimy and dilapidated by generations of financial neglect and overshadowed by proposals that they should be closed, deteriorate further as the recruitment of staff, particularly of nurses, becomes more difficult.

In Britain the ambitions of the 1960s have been tarnished by the failure of local authorities to fulfil their obligations to the vulnerable homeless¹ and by the failure to provide psychotic patients with the treatment they need^{2,3}. In 1985, even the Lord Chief Justice of England, Lord Lane, voiced his anger and frustration "to anyone prepared to listen".

The situation constantly deteriorates; the judgement in *Regina v. Hallstrom* exemplifies the unexpected difficulties in providing psychiatric care under the 1983 Mental Health Act: it is no longer lawful to insist on medication for patients on extended leave from hospital, while in-patient treatment is impeded because extra resources were not provided to meet the time-consuming administrative duties laid upon physicians by the 1983 Mental Health Act.

The essence of the new community care may be epitomized thus: "Social conditions may be thought of as leading to stress, which in turn leads to psychiatric symptoms . . . all organized attempts by the community to alleviate socially undesirable conditions may be considered part of community mental health. This may include poverty programs, head start programs, legal aid, planned parenthood,

vocational guidance, consumer information, and other community action programs . . ." (ref. 4).

The role of the psychiatrist almost vanishes in this optimistic formula for the communal alleviation of mental illness. The apathy and withdrawal that characterize chronic schizophrenia bewilders and frustrates those well-intentioned carers who had presumed that such symptoms would abate if patients were removed from hospital. Worse, those suffering from paranoid delusions, hallucinations and manic excitement are hardly likely to respond to consumer information campaigns or benefit from 'head start' programmes.

In our eagerness to repair the effects of the competition on which our society is based, and to treat the whole person and not just his illness, we must be careful not to deflect resources in such a way that we cannot treat severe illnesses. In Britain, in the name of community care, patients have been moved from Banstead Hospital

to Horton, which is itself to be closed, and from St Wulstan's to Powick, chosen as the first hospital in Britain to be closed. Yet 'trans-institutionalization' is probably the saving grace for the most seriously ill, but is far removed from the declared intention of locating patients closer to their original homes.

With 95,000 long-stay psychiatric patients discharged in England and Wales since 1954, it is surprising that only 4,000 of them can be found in local authority care — the same local authorities anxious to see the hospitals closed entirely — while common lodging houses, cheap accommodation and reception centres are rapidly disappearing⁵. Yet few local authorities, including 34 London boroughs, have fulfilled the modest guidelines for day centres in the 1975 White Paper *Care in the Community*⁵. Instead they are engaged on active retrenchment, particularly in London.

This cannot be an auspicious time simultaneously to close 55 psychiatric

IMAGE
UNAVAILABLE
FOR COPYRIGHT
REASONS

Out in the community or out in the cold? A resident of one of London's short-stay hostels.

hospitals in England. That figure is the government's estimate; The National Schizophrenia Fellowship estimates that 77 hospitals are to be shut. Can it be mere coincidence that the British Government is also planning to open 26 new prisons, one on the site of the recently closed Banstead psychiatric hospital?

As it happens, about a third of prisoners were found to be psychiatrically unwell on the day they were examined, both in England⁶ and in Scotland (R.S. Bluglass, personal communication). There is a significant inverse correlation between psychiatric bed occupancy and the numbers in prison in Europe, and, between 1950 and 1987, in England and Wales⁷ (correlation coefficient = -0.95 , $P < 0.001$) — see Fig. 1. There is also a highly significant relationship ($P < 0.001$) between serious psychiatric morbidity and imprisonment among the destitute^{2,5,7}. Yet the appalling situation of prisoners, who may spend up to 23 hours a day in solitary confinement without occupation, does not even make financial sense; the cost of maintaining a prisoner in Brixton gaol is £20,500 per annum and is £29,000 in a dispersal prison, compared with some £13,000 in a psychiatric hospital.

The planning of psychiatric care is complicated by the debate on what constitutes a psychiatric case. This again rests on the further question, a case for *what*? Medical or social treatment, prevention, aetiology, research or epidemiology? Some believe there is a continuum of increasing distress, with no boundary between distress and illness. That view accords with the over-inclusive and nebulous aspirations of the World Health Organization (WHO), which defines health as "a state of complete physical and mental well-being, and not merely the absence of disease and infirmity". Others, in accord with medical diagnosis, recognize distinct psychiatric disease entities, based on syndrome clusters.

One pragmatic approach is to define a psychiatric case by a person's ability to cross certain thresholds to get to psychiatric services, either by direct self-referral or referral by a general practitioner. This approach will include in the psychiatric ambit those with self-imposed problems, such as alcohol and drug abuse, and has implicit in it the danger that deviant behaviour might define a case — thus fueling the pungent criticisms of psychiatry of Szasz⁸, and the conspiratorial views emphasized by sociologists and social workers.

The 'threshold' approach has the further disadvantage of being a demanded definition. It fails to distinguish the vociferous preferences of those with mild problems from the muted distress of those with severe problems; and it overlooks the hidden iceberg of serious psychopathology in the prisons, among the destitute and in

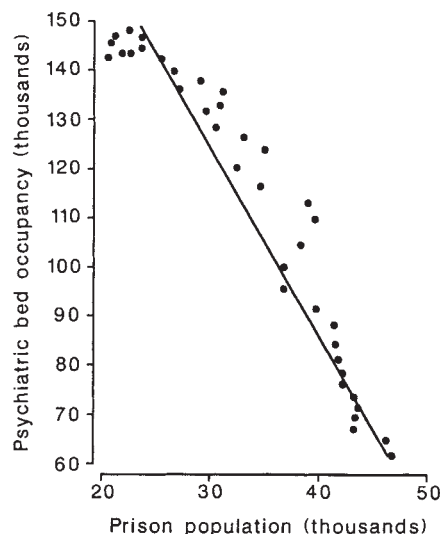


FIG. 1 Relationship between numbers of psychiatric beds occupied and the average daily prison population in England and Wales, 1950–1987. Best-fit regression line $r = -0.95$, $P < 0.001$.

the houses of 'caring' landladies who, in my experience, may not speak English, yet are still the fragile sheet-anchor of local authority commitment to community care.

In some surveys, up to 95 per cent of mild cases recover spontaneously, two thirds after six months. Those who fail to do so are often beset by persistent social problems and lack of support, implying social rather than psychiatric solutions. Yet the demand for psychiatric services encompasses these mild cases involving social malaise, who already engage a considerable proportion of specialist services, but who can be helped by a variety of non-psychiatric agencies.

Questions of cost and the deflection of limited resources apart, it would seem innocuous that physicians should respond to personal difficulties in adverse circumstances, seeking to relieve attendant unhappiness, frustration, dissatisfaction and demoralization. But doing so attracts the criticism that psychiatrists are 'medicalizing' distress to serve their own interests; and it increases the misunderstanding of what constitutes psychiatric illness, thus deflecting resources from those with severe psychopathological conditions.

A further danger when physicians embrace such personal problems is that of creating a lifelong, if intermittent, 'patient', one who may be all too ready to advance his or her condition to abrogate responsibility. Promoting illness behaviour and fostering dependency and social incompetence are considerable hazards. 'Crisis intervention' especially seems to run this risk — for example, that domestic disharmony may be construed as psychiatric illness.

The well intentioned response of medical practitioners to the personal pro-

blems that confront them often does some good. But the understandable search for the social determinants of distress cannot be mistaken for a complete explanation of the psychoses. Yet that is what it is taken to be by some anti-psychiatry lobbyists⁸, especially when evidence for a biological component accumulates (for example, see ref. 9).

Social workers, counsellors and psychologists feel competent to respond to minor problems, with the result that 14 per cent of the British population receives some form of psychiatric help. Social workers are able to support and counsel cases of mild depression and anxiety, particularly when they also address themselves to practical issues¹⁰, which, despite the kernel concept of community mental health, they are often disinclined or ill-equipped to tackle. Instead they prefer 'case work', a popular term for psychotherapy in social work circles.

Few psychotherapists would now argue that their skills are the exclusive domain of medically qualified practitioners, but medical qualifications are attractive to the aspirant patient. Indeed status, assured confidentiality and a trust in professional standards, together with kindly reassurance, are probably significant components in the nonspecific support that forms such an important part of the psychotherapeutic transaction.

Some psychiatrists, mostly those with predominantly psychotherapeutic interests, further confuse the distinction between (medically qualified) psychiatrists, psychologists and psychotherapists (it is still possible, in Britain, for anyone to call themselves by either of the last two terms without qualifications or training) and emphasize neuroses and personality problems to the neglect of the psychoses and organic brain syndromes. The undoubted educational benefit to general medical and psychiatric trainees of qualified psychotherapy supervision requires cases suitable for supervision, adding to the impression that such cases demand medical treatment.

Yet, with tightly limited resources, the almost inevitable psychiatric attention that determined and articulate help-seekers will obtain is likely to lead to a corresponding reduction of care for the most severely ill¹¹. But whether this distressed group is 'ill' depends on the definition; their pressing social problems, the likelihood that their distress will be spontaneously resolved and their suitability for psychotherapy should not crowd out the needs of psychotics and the chronically impaired, whose social problems are secondary. People with colds may be ill, but people with pneumonia should not be neglected in providing for them.

In the urgent planning for the closure during a short time of perhaps as many as 77 psychiatric hospitals in England and

Wales, the needs of the distressed, as distinct from the sick, assume an unwarranted prominence. Indeed the condemnation of the psychiatric hospitals is fuelled by the desire of these distressed people for more attractive and socially acceptable facilities, sequestered from those with undoubted illnesses. The fear of many community centres' personnel in the United States is that 'bad' patients may drive out the 'good'. Thus the psychotic are implicitly or explicitly excluded — partly because of their dishevelled appearance and social ineptitude, and partly because their illnesses are unremittingly chronic and provide little reward for therapists who like to see their patients improve.

Is it even possible that on society's hidden agenda there is a tacit understanding that health care resources should generally be concentrated on maintaining active members of society? Perhaps it is agreed that a temporary set-back to productive activity should be dealt with swiftly and effectively, taking priority over the needs of those with irremediable problems. The reality of these assumptions is debatable, but the debate is usually considered inappropriate and is avoided.

Nevertheless, the conduct of affairs is compatible with the sinister hidden assumption. If a person is a danger to society, some form of containment will be found; but if he or she is consumed by gross inadequacies through chronic mental illness, very little may be done. On the other hand, persons with minor psychiatric problems who are enterprising and energetic in seeking help are more likely than not, in Britain, to obtain group and individual psychotherapy within the National Health Service. Certainly they have such opportunities in London, even though that is also where chronic problems are congregated and consequential destitution is most apparent^{4,7} — see Fig. 2.

Resources are unequally distributed. Those who already have are accruing and campaigning for more. "If a competitive process is at work in the allocation of treatment, it is likely to favour young, competent, motivated, successful people"¹¹ sometimes known as YAWIS — young, attractive, white and intelligent — zealously seeking a deeper insight into their unconscious motivations. The planning of future mental health care in Britain increasingly reflects the influence of this distressed but generally articulate middle-class group of psychotherapeutic



FIG. 2 London as a magnet for the dispossessed. The map shows the place of birth of 201 destitute people interviewed at Christmas and on New Year's Day in 1985 (□), 1986 (○) and 1988 (◇). (Dept Medical Illustration, Royal Free Hospital.)

sophisticates, who are preoccupied with the discomforts of the worried well and are living in a cosy symbiotic relationship with psychotherapists.

Facilities have been, and are being, disproportionately directed to their requirements¹¹ while the silent misery of the chronic schizophrenic is forgotten. But unless the needs of chronic psychiatric illness are amply met, there is a risk of violence², suicide⁵ and disease, including communicable diseases such as tuberculosis^{2,5} and imprisonment^{2,3,7}.

Meanwhile, huge numbers of psychotic people are struggling to survive outside hospital, with dwindling support from the social services, often none whatsoever. Unless access to the attractive community centres is restricted to the polite and well-attired, those facilities will quickly silt up with chronic patients, whose appearance and behaviour is erroneously thought to be the product of institutional care, but who will as readily leave ash and cigarette burns on new furniture and carpets as on old.

If personal calamities are to be avoided, access to the new facilities cannot be restricted on the grounds of appearance and behaviour, or by taking advantage of the uncomplaining apathetic indifference so often found in chronic schizophrenia. But that is what happens in community centres in the United States.

There is a crying need that attention should be directed first to the needs of the most severely sick, who make no demands

and raise no protest. It is chilling that, in the United States, where psychoanalysts have for long dominated psychiatric practice and where psychiatric hospital closures have reached worrying proportions in California and New York, two out of every five people suffering from psychosis never receive treatment¹².

The active policy in England and Wales of closing psychiatric hospitals is based on a simplistic notion of an indefinite linear extrapolation of an alleged five-year trend, inadequately supported by the figures. The policy emerged in the same year as Goffman's polemic *Asylums* and is enshrined in Mr Enoch Powell's Hospital Plan for England and Wales of 1962, in which the consequences of the extrapolation are stripped of all reasonable caveats.

This nonsense, although repeatedly shown to be so by events, continues to influence policy. Yet doubts have been expressed that there will be sufficient funds to provide for adequate care. Thus the Islington (London) Health Authority says

in its Draft Operational Plan for 1987/88 that "it cannot be emphasized enough that the district is not funded to provide a comprehensive local service ... and is not statutorily required to provide such a service". The North-East Thames Regional Health Authority's Strategic Advisory Group has recently proposed a policy of reduced expenditure on those districts with most patients, describing the policy as "retrenchment and reprovision" and observing that the "unstoppable juggernaut" of hospital closure "merely arising out of the Government's wishes" cannot be halted. Meanwhile loneliness, degradation and destitution among the psychiatrically ill are rampant^{5,7}.

On Christmas Eve in 1988, out of a hundred people we interviewed in London at 'Crisis at Christmas', a temporary shelter providing food, medical attention and recreation over the Christmas period, one in five had positive symptoms of schizophrenia; one in three had definite or probable schizophrenia and two in five were psychotic at the time of interview (42 per cent of those permitting an examination of their mental state). Twelve of the psychotic respondents (13.6 per cent) had never been in contact with psychiatric services, ten (32.3 per cent) were not receiving any benefit entitlement and 25 (80.6 per cent) had been to prison, including one who had been sentenced to confinement at the special hospital at Broadmoor for attempted murder.

More than half the sample interviewed

were either psychotic or had previously received in-patient psychiatric treatment (including a convicted murderer). And *pace* the Resource Allocation Working Party (RAWP) formula (by which medical resources are taken from London and applied to the provinces), more than 90 per cent had migrated to London from elsewhere (Fig. 2).

Separation from supporting networks is characteristic of psychiatric illness, particularly schizophrenia: 64 per cent of the destitute sample claimed to have neither friend nor acquaintance, 36 per cent were not receiving social security benefits of any kind and 63 per cent had slept rough the previous night, a deterioration from the previous year, which nevertheless revealed a similar pattern⁵.

On New Year's Eve 1988, 44 per cent of the destitute we interviewed were actively psychotic, and a further 16 per cent had been discharged from psychiatric hospitals after treatment for psychoses⁷.

It should come as no surprise that community 'care' leads to disease and death⁸. As for civil liberties and freedom of choice, it is surely relevant that only 19 of the 450 long-stay patients now at Friern Hospital, north of London, are there by compulsion, and that 16 of them are constrained by court orders as an alternative to being in prison or a prison hospital. Nor may it be surprising that, before the transfer of funds from the National Health Service to the social services of local authorities in respect of community care, social services used to express an exquisite concern not to relocate those who did not earnestly seek to leave the hospital, and that this concern has now been replaced by an enthusiasm to close hospitals entirely.

Despite high staffing levels the social services are ill equipped to cope with the problems of severe illness because so many of the staff are unskilled¹³, while the financial formula by which the care is provided is a recipe for disaster. Yet only

the most disturbed patients are left behind, thrown together in blighted hospitals unable to recruit staff.

Ironically, despite financial difficulties these same hospitals have a wide range of facilities and amenities; occupational and industrial therapy; music, art and pottery; psychotherapy, social skills and relaxation classes; specialist diets under the jurisdiction of dieticians; religious services; patient clubs and cafeterias; gymnasiums and remedial gymnasts; specialist services, such as dentistry, chiropody, ophthalmology and physiotherapy, which patients often fail to utilize in other settings^{5,7}, and organized recreational activities, holidays and outings. Often, these facilities are to be found in tempting, spacious and well-set-out grounds. Despite the prejudicial connotations of a 'total institution', the hospital communities are vital and caring, and are under constant external scrutiny.

In our planning for the care of patients at these surviving hospitals, which are havens that provide asylum from wanton cruelty and neglect^{2,5,7}, little thought is given to those very ill patients who are not looked after anywhere at all. We have learned from recent experience that community care can meet the articulate demands of the least needful, and we are repeatedly invited to regard such programmes as a mark of bold and imaginative planning. When will we turn our attention to the needs of those for whom the community does not care and who should be our first priority? □

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Another red herring leads nowhere

Why, these days, are so many brave new ideas destined to bite the dust? And why are so many of them in the physical sciences, supposedly the more exact, rather than in biology?

THE fate of cold fusion remains in doubt, but does not look bright. The fifth force, the notion that newtonian gravitational attraction is modified by a short-range force dependent on the composition of materials and which might be mediated by one of the missing particles of high-energy physics, has ceased to attract excitement. Now the latest casualty may be the idea that the observation of positrons with well-defined energy in the collision of heavy ions might similarly be the signature of one of the missing particles.

The first observations were made six years ago at the West German heavy-ion accelerator laboratory at Darmstadt, during measurements by a US-German collaboration of the products of the collision of uranium and curium ions (J. Schweppe *et al.* *Phys. Rev. Lett.* **51**, 2261; 1983). One of the objectives of the experiments was to search for "islands of stability" in the periodic table at atomic numbers much greater than those of even the artificial transuranium elements.

The surprise, instead, was a clutch of positrons with an energy of 320 keV crammed into a narrow range of energy spread over 75 keV. The mystery was sharpened (T. Cowan *et al.* *Phys. Rev. Lett.* **54**, 1761; 1985) when an apparently identical positron line appeared among the collision products of uranium and thorium ions with each other, with themselves and with curium ions.

Speculation was constrained, as it has been almost ever since. Daring possibilities were raised only to be dismissed. That the positrons might come from the radioactive decay of some excited state of one or other of the colliding nucleons or from the nucleus which, briefly, would be their conjunction was ruled out once there were several nuclei in the field. The lifetime of the supposed compound nucleus (estimated at 10^{-20} seconds) was in any case too short to allow the narrowness of the peak observed. So might the positrons come from the disturbance of the electromagnetic vacuum by close nuclear collisions or even from "the two-body decay of a previously undetected particle"? The collaboration promised new experiments collecting evidence of the presumed accompanying electron would be sought.

A year later, the group at Darmstadt had reported both the discovery of the partner electrons and an estimate of the mass of the supposed neutral particle — something like 1.78 MeV (T. Cowan *et*

al. *Phys. Rev. Lett.* **56**, 44; 1986). But the measurements then available provoked an awkward question — the presumed mass could be anything between 1.5 MeV and 1.8 MeV, a large spread for such narrow electron and positron lines. Later that year, a Stanford-Berkeley collaboration reported its failure, at a parallel installation at Berkeley, to find the pairs of energetic photons into which a neutral particle with mass should also, less often, decay (W. E. Meyerhof *et al.* *Phys. Rev. Lett.* **57**, 2,139; 1986).

Soon afterwards, at Darmstadt and Berkeley, there were more surprises — in particular, the appearance of positron-electron pairs with energy different from that first observed. There seemed to be at least three distinct electron-positron lines, corresponding either to neutral particles with energies of 1.64 MeV, 1.77 MeV and 1.83 MeV or, alternatively, to a single neutral particle with a complicated way of shedding energy. The striking technical achievement of that period was a switch from positron to photon counting, which made it possible to pin down the presumed mass more accurately, to within a few keV either way — making the mystery more puzzling.

For the Stanford and Berkeley collaboration (now joined by people from Rochester and the Lawrence Livermore National Laboratory), growing complexity has evidently been frustrating. Somehow, the group persuaded the Lawrence Berkeley Laboratory to allow it seven weeks of run-time on Super-HILAC, the linear accelerator for heavy ions. For practical purposes, they have run through the experiments first reported in 1985, keeping to roughly the same energy of just about 6 MeV for each nucleon in the projectile nucleus. The statistics are inevitably much better than in any previous measurements.

And the result? The objective was to record the coincident emission of pairs of photons (γ -rays at this energy), to measure the energy of each of them and to equate the sum with the energy of the presumed neutral particle. For the collision of uranium projectiles with a thorium target, there are no fewer than five peaks between 900 keV and 1,000 keV, with others at greater energy.

But why so many peaks? Three peaks were bad enough, but, as Groucho Marx would have said, "five is ridiculous!" But the Stanford-Berkeley collaboration has

the neatest answer: the peaks in the energy of the photon-pairs have nothing at all to do with an unknown neutral particle, but arise simply because some heavy ions in collision are excited into high rotational states, perhaps with 30 quanta of rotational energy, from which they decay with the emission of photons (K. Danzmann *et al.* *Phys. Rev. Lett.* **62**, 2353; 1989).

This simple interpretation is convincing for reasons other than its simplicity. For heavy nuclei, the difference between successive rotational states is not constant (as it is for some molecules) because rotation flattens a nucleus, so that the single measurements of the slightly different energy of photons in an apparently coincident pair can be used to show that the measured peaks are consistent with being successive rotational de-excitations of a uranium nucleus. The equipment, luckily, also records the energy of many photons which are not apparently one of a pair; some of the peaks in that record correspond to the loss of a single rotational quantum from a highly excited state.

This interpretation does not prove that the positrons observed cannot arise from the decay of an unknown neutral particle, although the Stanford-Berkeley group say flatly that at least one of their peaks cannot be a two-body decay. More formally, they argue that if their photon-coincidences come from the decay of a neutral particle, the electron-positron peaks reported both from Darmstadt and (previously) Berkeley are almost certainly too small.

The positrons previously observed are, in all likelihood, secondary products of single γ -rays released from nuclei put into high rotational states by collision (which, counter-intuitively, requires that the collisions should be nearly head-on, which explains why they are rare).

There are no morals to be drawn from this tale. If neutral bosons were created in heavy-ion collisions, that would have been important, but it is also good to know the truth. That the search may have occupied a few hundred man-years of researchers' time, not to mention two of the world's most sophisticated accelerators off and on, is in the long run neither here nor there; those concerned have learned a lot of physics and have improved the state of the art. They also have the comfort of knowing that their working hypothesis began life as a well-founded puzzle. Who can ask for more?

John Maddox

Howard M. Temin

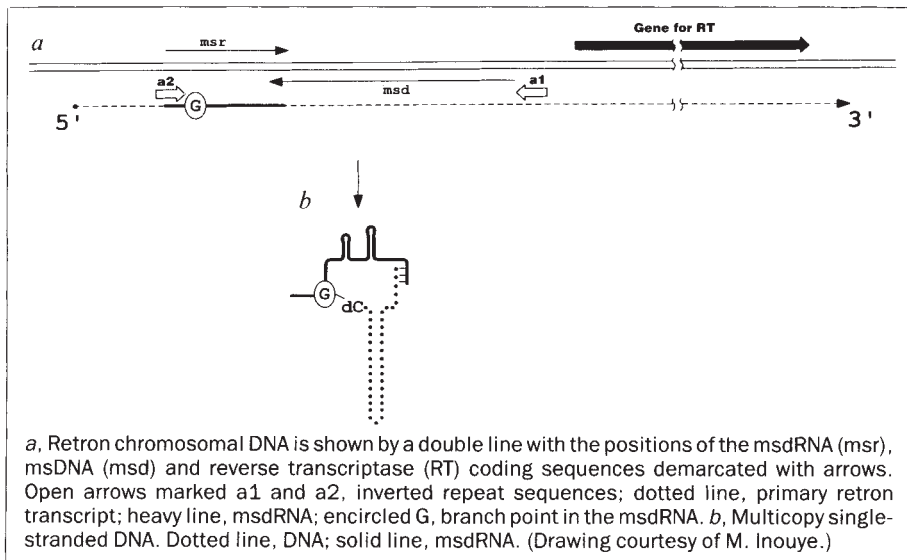
The common mode of information transfer in biological systems is DNA to

They called the satellite DNA ms (multi-copy single-stranded) DNA¹⁰. Similar msDNAs were found in other myxobacteria and, more recently, in 5 per cent of *E. coli* strains⁴. The msDNA contains covalently attached DNA and RNA overlapped for a few nucleotides at their 3' ends, a branched rG residue with a 2',5'-phosphodiester bond to DNA, and stem-loop structures (*b* in the figure)¹¹. Lim and

Reverse transcriptase and reverse transcription have now been found in most forms of life — bacteria, plants, animals and viruses. Some evolutionary biologists have suggested that there was an earlier RNA world, so that a reverse transcriptase activity would have been needed for a template-based switch to DNA-based life¹³. Thus, the bacterial retron reverse transcriptases could either be the descendants of an extremely ancient enzyme or they could have evolved from cellular DNA polymerases which in turn evolved from the first reverse transcriptases. The absence of the retrovirus and retrotransposon nucleoprotein particle-based reverse transcription in myxobacterial msDNA synthesis also indicates the antiquity of the bacterial retron.

So far, all reverse transcriptases have some amino-acid sequence identity, suggesting one evolutionary origin¹. However, the myxobacterial reverse transcriptases share with other myxobacterial genes a particular codon usage (high GC content), indicating that the myxobacterial reverse transcriptase gene coevolved with the rest of the myxobacterial genes³. The *E. coli* genes, by contrast, are more recent in *E. coli*. They are in only 5 per cent of *E. coli* strains and have different codon usage from other *E. coli* genes. Furthermore, there is great variability in nucleotide sequence among the reverse transcriptase genes seen in *E. coli* chromosomal DNA^{1,4,5}.

Both bacterial and retroviral systems share the characteristic that the strand encoding the reverse transcriptase is also the template strand for reverse transcription. The bacterial template RNA also apparently serves as the primer for reverse transcription (which involves the 2'-hydroxyl of rG), whereas retroviruses use a captured cellular transfer RNA as primer. In myxobacterial reverse transcription there is coordinate RNaseH activity, whereas RNaseH acts separately



The discovery of the bacterial reverse transcriptase began when a rapidly renaturing fraction of DNA was found when Yee and Inouye were studying the size of the DNA of *Mycococcus xanthus* and another myxobacterium *Stigmatella aurantiaca*⁹. These bacteria were of interest because of their ability to differentiate. In 1984, Yee *et al.* reported that the rapidly renaturing DNA in these bacteria can be found in a satellite band in routine preparative acrylamide gel electrophoresis.

Both groups have analysed the msDNA and the genes encoding it. Coding of msDNA involves chromosomal loci for the DNA and RNA parts on opposite strands of the chromosomal DNA with the 3' ends of the coding sequences for the msDNA and msdRNA overlapping. However, the msdRNA does not have genotype and phenotype like some RNAs (such as ribozymes), and a nearby open reading frame is required for the formation of msDNA (see part *a* in the figure). This whole element is called a retron (see table). The open reading frame of the retron has sequence similarity to the genes for other reverse transcriptases. In particular, it has a conserved Tyr-X-Asp-Asp sequence (but not a nearby Lys residue

Retroelements and retrosequences: DNA encoding reverse transcriptase or resulting from it

Name	Reverse transcriptase	Transposition/integration	LTR	Virions
Retron	+	0	0	0
Retroposon	+	+	0	0
Retrotransposon	+	+	+	0
Retrovirus	+	+	+	+
Pararetrovirus	+	0	+	+
Retrosequence	0	0	0	0

Names and properties of DNA sequences encoding reverse transcriptase (retroelements) or resulting from reverse transcription (retrosequences and all retroelements except retrons). (Usage is different from that of refs 6, 7.) LTRs are long terminal repeats, or other structures leading to terminally redundant element RNA. Defective forms of each retroelement and retrosequences, except the retron, have also been found.

Emilio Segrè (1905–1989)

in retrovirus replication. In retroviruses, the RNaseH activity is encoded as part of the gene for reverse transcriptase. In myxobacterial retrons, there are no apparent RNaseH-coding sequences. Perhaps the DNA polymerase and RNaseH activities have not yet diverged from each other. Interestingly, it was reported (M.T.R. Kuiper, University of Ohio, Columbus) at a recent meeting* that the Mauricéville *Neurospora* mitochondrial plasmid reverse transcriptase uses as a template an RNA with transfer RNA-like sequences at its 3' end. Some plant viral RNAs have a similar 3' end.

If we accept the argument for the antiquity of the myxobacterial reverse transcriptase gene, then the precursor of this gene was the ancestor of all present-day retroelements (see table). Thus, the path of evolution went from retrons with reverse transcriptase to retrotransposons with the ability to transpose to retrotransposons with long terminal repeats to retroviruses with the ability to form virus particles to pararetroviruses which have lost the ability to integrate/transpose and perhaps, as was speculated at the meeting and elsewhere¹⁴, to some circular DNA viruses.

What is the role of cellular reverse transcriptases? There have been several suggestions that reverse transcription plays a role somehow advantageous to cellular organisms^{15–19}. However, despite much work, all previously described reverse transcriptases seem primarily of benefit for the gene for reverse transcriptase itself or the retroelement that encodes it and not for the cellular organism containing the reverse transcriptase gene. Thus, there are many retroelements including hepadnaviruses, caulimoviruses, retroviruses, retrotransposons and retrotransposons (or, as I originally called them, viruses and proviruses¹⁵) which are parasitic and whose main physiological role relative to cellular organisms is their own self-reproduction. But there is as yet no clear evidence of an advantage for cellular organisms in expressing reverse transcriptase. However, the myxobacterial and *E. coli* reverse transcriptase genes appear to be single-copy, indicating that their function is not to amplify the retron relative to other bacterial genes; that is, the retron is not 'selfish' DNA.

One thing that is clear in considering possible functions of the bacterial retrons is that the synthesis of msDNA from an RNA template ensures that the sequence of msDNA is hypervariable. Could this be a clue to its function? Why would it be of value to some bacteria to have conserved through evolution an ability to synthesize multiple copies of a small hypervariable piece of DNA? Or rather is msDNA a convenient primer for the copying of useful mRNAs and subsequent gene

EMILIO SEGRÈ, the eminent Italian nuclear scientist who shared the 1959 Nobel Prize for physics for the creation of antiprotons, died near his home in Lafayette, California, on 22 April 1989.

Although best remembered for his nuclear research, Segrè's early work was in atomic physics. During 1930–32 he performed experimental studies of forbidden lines in atomic spectra and their Zeeman effect. A study of the Stark effect on the highly



Segrè (centre) with Enrico Fermi (left) and Hideki Yukawa (right) in 1948.

excited states of alkaline atoms, in which I collaborated, led to the discovery of energy shifts in these levels attributable to the presence of foreign gases. Fermi's theory of this phenomenon in 1933 represents the first recognition of the peculiar properties of the states which are nowadays termed Rydberg states. Segrè and Fermi also collaborated on a theory of the hyperfine structure of atomic lines.

Segrè changed to nuclear physics in 1934, working in Fermi's group at the University of Rome. He participated in the first investigations of the artificial radioisotopes produced by neutron bombardment of uranium and thorium; in the discovery of slow neutrons; and in the discovery of the anomalously large cross-sections for the absorption of slow neutrons by medium and heavy nuclei. The group also showed that slow neutrons can reach thermal

energies, a process to become important in fission studies. Later, with Perrier at the University of Palermo, Segrè discovered a few long-lived isotopes of the missing element with atomic number 43. This element, produced in proton-bombarded molybdenum, they later named technetium.

Having emigrated to the United States (settling in Berkeley) after the promulgation of antisemitic laws by the Italian fascist government in 1938, Segrè identified a metastable isomeric state of technetium-99 with a half-life of 6 hours which became one of the mainstays of nuclear medicine.

From 1943 to 1946, Segrè took part in the Manhattan project at Los Alamos, leading an experimental team investigating the spontaneous fission of uranium and plutonium. Returning to Berkeley in 1946, he studied proton-neutron and proton-proton scattering using polarized and unpolarized beams of protons. Later work led to the Nobel prizewinning discovery of the antiproton in collisions of protons with nuclei at the Bevatron accelerator at Berkeley.

This was done using a large mass spectrometer, built by Segrè, Chamberlain, Wiegand and Ypsilantis, which demonstrated the existence of a particle with the mass of the proton and negative, not positive, charge. My group and that of Goldhaber joined Segrè in studies in which nuclear photographic emulsions were bombarded by a beam of antiprotons selected by the mass spectrometer. Nuclear 'star' patterns evident in the developed emulsions showed all the features expected for the annihilation of antiprotons, as defined by Dirac's theory of particles and antiparticles and an empirical knowledge of nuclear particles.

Segrè's books on nuclear physics, *Experimental Nuclear Physics*, which he edited, and *Nuclei and Particles* are classics of the subject. Also, his recollections of his mentor, *Enrico Fermi, Physicist* and his two histories of physics, *From X-rays to Quarks* and *From Falling Bodies to Radio Waves* are highly popular.

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conversion¹⁹? Does the reverse transcriptase story still have more surprises to come? Almost certainly. There are still *Archaeobacteria* to be studied, and the telomerase from *Tetrahymena* contains essential RNA²⁰. Furthermore, AIDS ensures that reverse transcriptase will be in the news for a long time to come. □

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* *Molecular Biology of Retroviral Viruses and Elements* EMBO Workshop, Flumserberg, Switzerland, 3–7 April 1989.

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The dark cloud revisited

Cedric Lacey and Joseph Silk

INTERGALACTIC clouds are believed to be common in the remote Universe, but nearby examples are exceedingly rare. The best example of a large intergalactic cloud that is sufficiently close for there to have been extensive multifrequency studies is in the M96 group of galaxies towards Leo at a distance of about 10 megaparsecs. Stephen Schneider and collaborators¹ have now completed a detailed survey of the Leo intergalactic cloud. Their results, reviewed below, enable us to discuss whether such a cloud may indeed be a prototype of the intergalactic clouds that are studied at large redshift in absorption towards quasars.

The Leo cloud was originally discovered, by Schneider and co-workers², by means of its radio emission at 21-cm wavelength from atomic hydrogen (H I). There has since been a possible detection of ionized hydrogen (H II) in the cloud. However, searches for emission of visible light from the cloud have failed to find any evidence for an accompanying population of stars. Searches for emission from interstellar molecules and dust have similarly been negative. The Leo cloud is thus remarkable for an object of its mass in its very low level of star formation activity relative to the amount of gas present. For this same reason, other objects of this type will be difficult to find, but may constitute an important population in the Universe.

Ring structure

The 21-cm survey, performed at Arecibo and at the Very Large Array, has revealed that the H I gas in the Leo intergalactic cloud is distributed non-uniformly around a moderately eccentric ring of diameter 200 kiloparsecs (kpc); for comparison, the diameter of our Galaxy is 30 kpc. The ring's kinematics are those of a keplerian orbit of period 4×10^9 yr, whose centre coincides in position and velocity with the centroid of the M105/NGC3384 galaxy pair. Interestingly, the mass-to-light ratio for the pair implied by this is only $25M_{\odot}/L_{\odot}$ ($M_{\odot}$ and $L_{\odot}$ are the solar mass and luminosity), comparable to the value one expects for a purely stellar population, suggesting that there is little if any mass in a dark halo around the galaxies, and in contrast to what is found in many spiral galaxies, including our own. The total H I mass amounts to $2 \times 10^9 M_{\odot}$, and the mean density is only of the order of 10^{-3} cm^{-3} . However, high-resolution observations³ reveal that about half the H I is in clumps of size a few kiloparsecs, mass around $10^7 M_{\odot}$, velocity width about 20 km s^{-1} and central density 0.1 cm^{-3} . The virial masses of the clumps (deduced from their dynamics) seem to

exceed the H I masses by factors of 2–4; if this discrepancy is real, the balance could be supplied by envelopes of H II. The gravitational field of the central galaxy pair will tidally disrupt any gas cloud with a density less than 0.1 cm^{-3} , so the clumps appear to be marginally tidally stable.

A high-sensitivity search has been made for H α emission in the red spectral region from ionized hydrogen, with a sensitivity to an emission measure of about $1 \text{ cm}^{-6} \text{ pc}$ for gas at 10^4 K , tentative evidence for a detection at this level in the most dense parts of the cloud being reported⁴. The ionization rate required to account for the observed H α intensity is substantial, amounting to some 6×10^5 hydrogen ionizations per second per square-centimetre column through the clouds. If the H II gas fills most of the volume of the cloud, then its inferred mass exceeds that of the H I. If the gas is photoionized by an extragalactic ultraviolet background, the inferred ionizing flux is only a factor of 2–3 below what one infers by extrapolating from observational upper limits on this background in the 1,400–1,900-Å range⁵.

It is also possible that the ionization is produced by the ultraviolet hydrogen Lyman continuum radiation from OB stars, which might be expected to form in the more dense clumps of H I that are comparable in mass and mean density to dwarf galaxies. Sensitive optical observations of one of the most dense clumps show that the maximum surface brightness in the visible-band is $27.7 \text{ mag arcsec}^{-2}$. For comparison, note that for the galactic disk in the solar neighbourhood, the ionizing flux is estimated⁶ to be $3 \times 10^7 \text{ cm}^{-2} \text{ s}^{-1}$ for a visible-band surface brightness of $23.5 \text{ mag arcsec}^{-2}$ (ref. 7; brightness increases with decreasing magnitude). Assuming conservatively that the stars in the clump have the same distribution over mass and age as in the solar neighbourhood, the surface brightness inferred from the ionization rate would be only $27.8 \text{ mag arcsec}^{-2}$, compatible with the quoted upper limit. Given the uncertainties in the stellar mass function and age of the population, it seems that stellar photoionization provides the most natural explanation of the H α flux.

The disruptive tidal field may provide the reason for the inefficient star formation, as it would inhibit clumping of the gas. The clumps which do exist would be vulnerable to disruption by energy input from young stars if star formation were too vigorous. The distribution of the ring material around a single keplerian orbit suggests that it originated as a single weakly bound object which was then tidally disrupted on close approach to the

central galaxy pair — the tidal field being about 10 times stronger at the pericentre of the orbit than at the apocentre. The alternative possibility, that the ring is a shell of ejected gas, can be rejected on account of its negligible radial expansion. The time for the cloud to fall into the system, assuming initial conditions moving with the Hubble expansion of the Universe, would be roughly $6 \times 10^9 \text{ yr}$, so the ring material might only have orbited once in the age of the Universe.

Longevity

The ring owes its longevity to the relatively high angular momentum of the material in it: if it were on a more eccentric orbit, it might by now have been accreted onto the central galaxies. The existence of such material is a natural consequence of the tidal origin of galactic angular momentum in the gravitational-instability theory of galaxy formation, in which neighbouring condensations spin up one another. The dispersion in the angular momentum at fixed mass is predicted to be large⁸, and should result in some galaxies having reservoirs of outlying gas with high angular momentum.

One expects intergalactic clouds within groups of galaxies to be more common at earlier epochs, because the process of collecting material into galaxies would not have been 100 per cent efficient. This material would only gradually be exhausted as encounters occurred between clouds and galaxies. This late accretion of metal-poor gas could play a role in galactic chemical evolution. It is also tempting to speculate that some of the absorption lines seen in quasar spectra are produced by intergalactic clouds similar to that in Leo. The average H I column density seen in the cloud is a few 10^{18} cm^{-2} , comparable to that in metal-line absorption systems, and the velocity widths are also comparable⁹. The expected frequency of absorption lines would also turn out to be about right if most galaxy groups at large redshift contained clouds like that in Leo. Perhaps the dark H I cloud in Leo is a prototype of a more extensive population of intergalactic clouds that played a significant role in the early Universe. □

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NOISE

Humpty Dumpty to Moslem art

W. Schleich and P.V.E. McClintock

HIGH-PRECISION measurements of gravitational-wave-induced length changes in Michelson interferometers, quantum-optical realizations of the Einstein–Bohr dialogue on Young’s double-slit experiment using micromasers, bistable systems, hydrodynamical pattern formation and magically complex phase-space webs with the beauty of Moslem art born out of non-linear dynamical systems — one could hardly name fields of physics further apart than these. Nevertheless, there exist features common to these seemingly unrelated topics, features that were the subject of a recent meeting*. All the

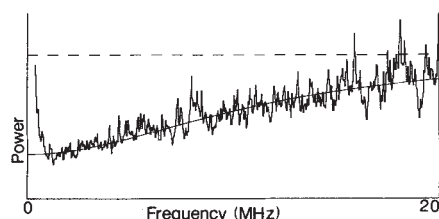


FIG. 1 Noise reduction factor R as a function of the frequency Ω for the intensity difference between the two beams in the optical parametric oscillator experiment of C. Fabre *et al.*. The dashed, horizontal line at unity corresponds to the vacuum level; the jagged, experimental curve, in good agreement with the solid theoretical curve, shows 69 per cent noise reduction around 2 MHz. (From T. Debuisschert *et al.* *Quantum Optics*; in the press.)

systems are affected in one way or another by noise or chaos: quantum noise in all its modifications, quenched, squeezed, inhibited or enhanced; noise as a factor in quantum measurements causing the irreversible destruction of interference fringes, a fate compared by J. Schwinger and colleagues to the irreversible one of Humpty Dumpty falling off the wall; classical noise triggering the switching of a bistable system; and the deterministic analogue of noise — chaos — arising in nonlinear systems.

Noise is ubiquitous in physical systems, generally being regarded as a nuisance. But increasingly it is becoming the subject of studies in its own right: as an aspect of chaos in dynamical systems; and as an aspect of the uncertainty principle and indeterminism in quantum systems. As it becomes better understood, long-standing problems in diverse topics are being solved and subtle new approaches to noise suppression are being devised.

The latter benefit can be illustrated by developments in the search for astrophysical gravitational waves. These are supposed to leave their signature in relative changes of length as little as 10^{-21} of heavy, aluminium ‘Weber’ bars or in tiny

shifts of interference fringes in a Michelson interferometer. The zero-point oscillations of the Weber cylinder or of the electromagnetic field, which tend to mask the effect, have consequently stimulated new discussions in the quantum theory of measurement and have boosted studies of nonclassical radiation fields, such as squeezed states (see the News and Views articles by D.F. Walls in *Nature* **324**, 210–211; 1986; and R.K. Bullough in *Nature* **333**, 601–602; 1988).

The ultimate aim is to follow a quantum system, such as a Weber bar oscillator, in time by making repeated measurements on it at discrete intervals, and to deduce from its evolution, or ‘trajectory’, the (gravitational) force acting on it. But what does it mean, to make a measurement? An answer is offered in the following procedure: start from a quantum system such as a free particle driven by a short pulse — the gravitational wave; couple it at discrete times to a heavy detector — a meter to follow its motion; and apply a modified Dirac–von Neumann wavefunction-reduction hypothesis, according to which a measurement of a meter variable reduces the *combined* wavefunction of system and meter to a pure wavefunction of the system alone. The meter variable is no longer a dynamical variable but solely a parameter. These are the ingredients of W.E. Lamb’s (Univ. Arizona, Tucson) operational prescription for sequential measurements on a quantum particle. According to newtonian physics, the particle initially at rest moves with constant velocity after the action of the gravitational pulse. Although quantum mechanics rejects the notion of such well defined trajectories, the result of the quantum version of this problem, evaluated according to the above recipe, is very similar so that one can determine the smallest detectable gravitational force.

The subtle relationship between noise and quantum measurement can also be illustrated by lessons learnt from quantum optics. An oscillator in a coherent state,

such as light from a laser, has its zero-point fluctuations — reflecting the Heisenberg uncertainty — distributed equally over the two conjugate variables electric field, E , and magnetic field, H . But why distribute them equally? Why not squeeze the fluctuations from E into H , or vice versa? An experiment measuring only the variable of reduced, that is, squeezed fluctuations must be superior to the corresponding one tackling the coherent state. The experimental realization of such squeezed states — now obtained in many laboratories — relies (P.L. Knight, Imperial College) on non-linear optical processes such as four-wave mixing or the parametric oscillator. In the latter case, a photon of frequency 2ω creates two photons each of frequency ω . Because the twin photons are born out of a single photon, there exists a strong correlation between them, consequently reducing the quantum noise in the difference between the two beam intensities by as much as 69 per cent, as shown in Fig. 1 (C. Fabre, Ecole Normale Supérieure, Paris).

One- or two-photon ‘micromasers’ (S. Haroche, Yale Univ.) can allow intriguing tests (M. O. Scully, Univ. New Mexico) of the complementarity principle (according to which wave- and particle-like descriptions are complementary). Send an atom prepared in a coherent superposition of two quantum levels through two consecutive micromasers as shown in Fig. 2. In this way, transfer the population to two neighbouring levels. Is the initial coherence between the two atomic states as manifested in an oscillatory ionization signal — temporal interference — preserved? To elucidate this question, imagine starting from coherent cavity fields with a large mean number of photons. In such a coherent state the fluctuations in photon number are large. Hence the two photons emitted in the transfer process, and caught in the cavities, do not change the field state significantly. One cannot deduce information concerning the transfer by studying the cavity fields and hence the coherence is preserved giving rise to quantum beats. However, if the experiment is started from a state of well-defined photon number, it is possible to detect the

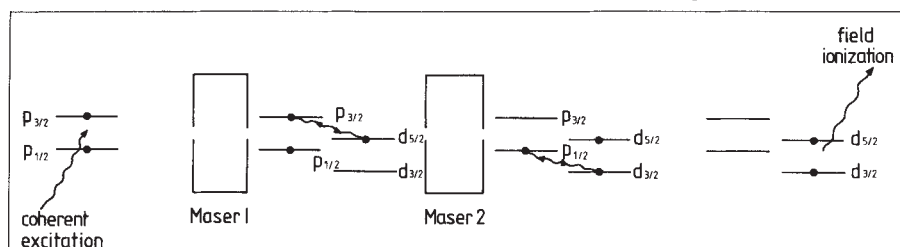


FIG. 2 The micromaser ‘welcher weg’ (‘which path’) detector of Scully and Walther. In passing through the first micromaser a Rb atom, prepared in a coherent superposition of $63p_{3/2}$ and $63p_{1/2}$ makes a transition from $63p_{3/2}$ to $61d_{5/2}$; and in the second, from $63p_{1/2}$ to $61d_{3/2}$. Depending on the maser state — coherent or number state — quantum beats detected via field ionization do or do not occur. (From M.O. Scully & H. Walther, *Phys. Rev. A*; in the press.)

*NATO Advanced Research Workshop on Noise and Chaos in Nonlinear Dynamical Systems, 7–11 March 1989, Turin, Italy.

increase, by one, in photon number induced by the population transfer. This destroys the coherence of the state, just as identifying which slit a photon passes through in the Young's double-slit experiment destroys the coherence and hence the interference fringes in that experiment. Bohr's standard explanation for the phenomenon involves arguments about the random phase of photons which, in this micromaser cavity experiment, are clearly inapplicable.

Classical studies of chaos have arisen as it becomes clear that noise is not necessarily a random, or stochastic, effect but arises from the complicated, but deterministic, dynamics of nonlinear systems. The paradigm for classical chaotic systems is turbulent flow in a fluid, which arises when nonlinear viscous effects dominate the smooth average velocity field of the fluid. A principal challenge is the calculation of the velocity fluctuations in fully developed turbulent flow, starting from the underlying equations of motion, the Navier-Stokes equations. It is the non-linearity (in viscosity) of these equations — the very source of the apparently stochastic noise — that has frustrated theoreticians from solving analytically the flow patterns, so that numerical solutions have been sought instead. But S. Grossmann (Philipps Univ., Marburg) has found a way of decomposing the velocity field into two parts, one representing the smooth average velocity field, the other the fluctuating turbulence (which depends on the scale length of the decomposition). This analytic approach should allow a better understanding of the features of turbulence.

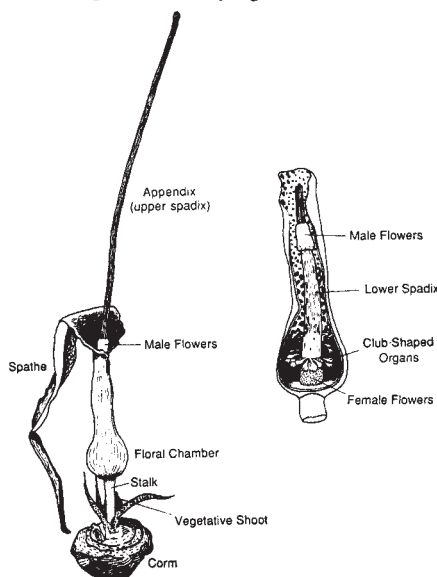
The phenomenon of stochastic resonance, used by Benzi *et al.* (*Tellus* **34**, 10; 1982) to try to account for a 10⁵-year periodicity in the Earth's ice-ages was beautifully demonstrated (R. Roy, Georgia Inst. Technology) in a ring laser and in analogue electronic simulations (F. Marchesoni, Univ. Perugia). This seemingly counterintuitive effect occurs in bistable systems where it enables small periodic signals to be enhanced, astonishingly, by the addition of extra noise. In effect, the added noise helps the much smaller (but coherent) periodic components to push the system over the central maximum in the bistable potential, and hence leads to amplification. In the case of the ring laser, it was convincingly demonstrated that, with a periodically modulated asymmetry between the clockwise and counter-clockwise waves, the signal/noise ratio in the output increases markedly when noise is injected into the system. □

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Hot sex in voodoo lilies

Jared M. Diamond

ALTHOUGH the use of metabolic heat to maintain body temperatures far above ambient is generally considered a hallmark of higher animals, particularly birds and mammals, it is also known to occur in flowers and inflorescences of at least six plant families, where a well-understood function of high temperature is to volatilize compounds that attract insect pollinators¹. *Arum* lilies such as skunk cabbage and the voodoo lily, for example, can increase the temperatures of their flowers in a brief burst by up to 22 °C above ambient. They thereby broadcast putrescent odours which have been compared with the smells of rotting flesh, decaying urine, faeces and



Inflorescence of the voodoo lily on D-day. Left, entire inflorescence; right, longitudinal cross section. (From ref. 4.)

sulphurous vapours², and which arise from volatile compounds including skatole, putrescine and ammonia^{2,3}. A new study of the voodoo lily (*Sauromatum guttatum*) by Raskin *et al.*⁴ now clarifies the triggering of heat production as well as revealing a second phase of heating with a separate function.

The voodoo lily's inflorescence consists of a trap-like floral chamber containing the female flowers and club-shaped organs, accessible only via an opening containing the male flowers. Surmounting the floral chamber is a slender rod resembling a car's radio antenna, termed the appendix (see cover illustration). Until the day of blooming (D-day), the appendix is concealed within a sheath called the spathe.

The trigger for heat production turns out to be salicylic acid⁵, whose concentration in the appendix begins to rise on the late afternoon of the day before D-day and peaks at 100 times the basal level. On

the morning of D-day, the spathe unfolds to expose the antenna-like appendix (see figure), in which salicylic acid stimulates a metabolic explosion by the cyanide-insensitive mitochondrial respiratory transport system⁶. The resulting metabolic rate rivals that of hummingbirds in flight. Production of heat, and hence of stench, peaks between 3 and 5 hours after dawn on D-day. By late afternoon, the appendix's temperature has dropped back to ambient and its salicylic acid content has declined to basal levels.

The timing of this first phase of heat production depends on surges in three factors: salicylic acid production, tissue sensitivity to salicylic acid and light exposure, which enhances tissue sensitivity to the acid still more. (Until the spathe unfolds, the inside of the inflorescence remains dark.) Of 33 analogues of salicylic acid tested by Raskin *et al.*⁴, the only ones that duplicate its stimulatory effect on heat production are acetyl-salicylic acid (aspirin), possibly because it is hydrolysed to salicylic acid, and the dihydroxy analogue of salicylic acid. The highest concentrations of salicylic acid are in the male and female flowers, but they do not produce heat. Thus, it remains unclear whether the acid is initially produced in the flowers and then transported to the heat-producing tissues of the inflorescence, or whether it is produced in the latter themselves.

The stench of the amines and indoles broadcast from the appendix lures insect pollinators to the floral chamber. From the night after D-day until dawn of the day after there is a second heating phase that differs from the first in originating between the male and female flowers in the centre of the floral chamber, producing less heat (temperatures 'only' 10 °C above ambient), and lasting longer (14 instead of 7 hours). Like phase 1, phase 2 is preceded and presumably triggered by a local 100-fold rise in levels of salicylic acid.

The heat of phase 2 stimulates activity of the insects attracted by the heat of phase 1 and trapped inside the flower chamber by the slippery concave walls and hedge of club-shaped organs. In addition, a sweet odour from the club-shaped organs may specifically stimulate insect mating behaviour. At the peak of phase 2, the male flowers shed their pollen into the

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hot floral chamber, where the bustling insects proceed to deposit pollen on the female flowers. The inflorescence then shrivels, permitting the insects to escape and cross-pollinate other inflorescences.

Devout scientists before Darwin would have cited this whole set of seemingly bizarre adaptations as evidence for a divine creator. In fact, the crucial ingredients of metabolic heat and volatile compounds

are ubiquitous in plants. Because a slight increase in metabolic heating would produce a graded advantage in attracting insect pollinators, it can now be seen how natural selection led to hot voodoo lilies without prior design. □

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CHEMICAL KINETICS

Doppler views of photofragments

Ian W. M. Smith

THE past few years have brought remarkable advances in the understanding of how molecules fall apart following absorption of a photon of radiation. A whole armoury of techniques, using all the extraordinary properties of laser radiation have been deployed to look at the subtleties of photodissociation: for example, how the rate varies from different excited states, how the excess energy is distributed between the degrees of freedom of the products, and how the motions of separating fragments are correlated. These results have provided extraordinary insight into the forces and torques which act between atoms and molecules at short range when the nature of the chemical bonding is altered suddenly as a result of a photon being absorbed. One general technique makes use of the Doppler effect to determine the amount of translational energy released. A particularly elegant version of this method is velocity-aligned Doppler spectroscopy, already used (C. Xu, B. Koplitz & C. Wittig *J. chem. Phys.* **87**, 1062–1069; 1987 and D. Baugh, B. Koplitz, Z. Xu & C. Wittig *J. chem. Phys.*

88, 879–887; 1988) to examine the photodissociation of some simple molecules, but only in a recent article (Z. Xu, B. Koplitz & C. Wittig *J. chem. Phys.* **90**, 2692–2702; 1989) has a full analysis of the method been given.

To appreciate the value of Wittig's experiments, it is useful to begin by explaining how laser spectroscopy can often be applied to determine the distribution of molecular fragments over internal (vibration-rotation) states. In these experiments, the pulsed laser causing photodissociation and the pulsed laser used to 'probe' the product's state distribution are fired essentially simultaneously so that the nascent distribution is observed rather than one modified by collisional redistribution. The tunable probe laser scans through the absorption spectrum of a product and populations in specific quantum states are deduced from the strength of individual spectral lines. The spectrum is usually recorded either by laser excitation spectroscopy (LES), that is by observing the total fluorescence emitted as the wavelength of the probe laser is scanned, or by measuring ions formed as a result of a resonantly enhanced multiphoton ionization (REMPI) process. In REMPI, one (or more) photons raises the molecule to a discrete excited state and from there absorption of one (or more) photons causes ionization.

Although many experiments on photodissociation have been carried out using LES or REMPI to characterize product states, the techniques cannot be applied universally, as many fragment species may not have sufficiently stable excited states within the range of tunable lasers, or even because the spectrum of a polyatomic fragment becomes too complex for analysis. One solution is to measure the translational energy yield and then to infer the distribution of internal energies from energy balance, the total energy available being defined by the frequency of the monochromatic photolysis laser and the dissociation energy of the molecule. The basic principle is that used in photoelectron spectroscopy: the distribution of electron kinetic energies is measured and structure

in this distribution is related to the production of molecular ions in different electronic and vibrational energy states.

The earliest forms of translational spectroscopy were based on time-of-flight methods (K. R. Wilson, *Discuss. Faraday Soc.* **44**, 234–236; 1967). A molecular beam was crossed with a pulsed laser (a 'photon beam') and measurements were made of the distribution of fragment flight times to a mass spectrometer detector. A fascinating variant of this technique has recently been introduced by Welge, Dixon and colleagues (J. Biesner *et al. J. chem. Phys.* **88**, 3607–3616; 1988) in investigations of the photodissociation of H_2O and NH_3 . The H atoms produced are ionized by a REMPI process induced by a probe laser fired at essentially the same time as the photolysis laser. As long as the concentration of H^+ ions is low enough to avoid effects due to Coulomb repulsion, the distribution of the H^+ flight times reflects the velocities of the H atoms before ionization and one avoids the inefficient process of ionizing neutrals at the entrance to a mass spectrometer.

In contrast, in Doppler spectroscopy, a narrow-linewidth laser is scanned through the profile of a single spectral transition whilst LES or REMPI signals are recorded: motion towards or away from the laser Doppler shifts the absorption frequency above or below the natural frequency. However, if the photolysis and probe lasers are fired simultaneously, a problem arises because then all photofragments are observed and Doppler spectroscopy reflects not a difference in molecular speeds but one in molecular velocities. For example, signals at zero Doppler shift arise from species with zero

100 years ago

FORMALDEHYDE, CH_2O or H.CO.H , the first member of the important series of aldehydes, has been synthesized by Prof. Jahn, of Cronstadt, Hungary, in a most instructive manner. During the course of Dr. Jahn's well-known researches upon the volumetric estimation of hydrogen by means of palladium, it was noticed that the presence of carbon monoxide always considerably disturbed the occlusion of the hydrogen by the palladium. As there was a possibility that some of the hydrogen had bodily united with the carbonic oxide with formation of formaldehyde, it was determined to repeat the experiment upon a larger scale. A mixture of carbon monoxide and hydrogen was therefore led over a layer of spongy palladium, and the products passed through a series of bulbs containing water. On detaching the bulbs the odour of aldehyde was perceived, and the contents at once reduced an ammoniacal silver nitrate solution with formation of the silver mirror characteristic of aldehydes. Hence it was quite evident that the carbon monoxide and hydrogen had partially united in the pores of the palladium with production of formaldehyde. From *Nature* **40**, 85; 23 May 1889.

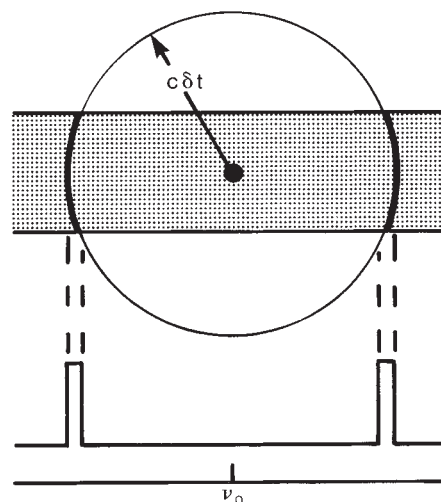


FIG. 1 Two-dimensional representation of a VADS experiment in which H atoms of a single speed c are produced and are detected after a delay δt at the central dark point. The shaded region indicates the photolysis region so only products initially formed on the heavily drawn arcs of the circle will be detected. This would give rise to the spectrum shown below. ν_0 , Natural transition frequency.

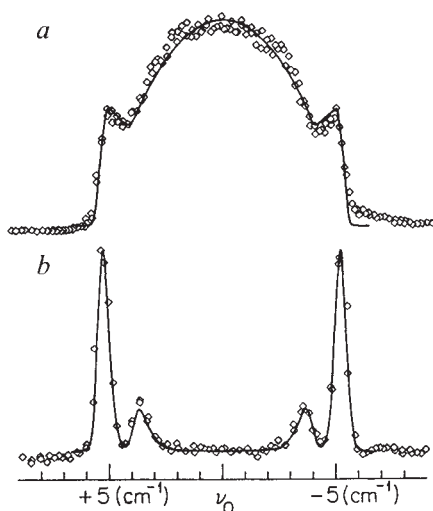


FIG. 2 Doppler profiles for H atoms produced by HI photodissociation at 248 nm probed at zero delay and at 320 ns delay. The presence of two peaks on each side of the line centre arises because the I-atom can be formed in two different electronic states.

component of velocity along the probe laser, however rapidly they are moving perpendicular to the laser beam.

Wittig's solution to this problem is elegant in its simplicity. In velocity-aligned Doppler spectroscopy (VADS) a delay is introduced between the collinear photolysis and probe lasers. The effect is to discriminate in favour of those photofragments created with velocities directed along the direction of the laser beams. The experiments have much in common with those of Welge's group. The photolysis and probe laser beams are again collinear and the probe laser causes ionization of the H atom products of photodissociation with one vacuum-ultraviolet photon exciting H atoms in the Lyman- α transition and a second photon at three times the wavelength ionizing the excited atoms. In the VADS experiments, however, using a repelling voltage and apertures, H⁺ ions formed in a small volume element are injected into a drift tube and then detected.

The situation when there is a time delay (δt) between photolysis and probing can be considered in terms of the simplified model represented in Fig. 1. Suppose that only ions at the centre (●) are detected and that all H atoms were originally created with the same speed, c . Then the only atoms present at the centre at time δt would be those which were originally generated on the surface of a sphere centred on the detector and with a radius $c\delta t$. Moreover, H atoms could only have been produced on those parts of the sphere's surface which are irradiated by the photolysis laser. Clearly, as δt is increased, the radius of the sphere also increases, and the fraction of the sphere's surface which meets the second criterion diminishes. This means that as δt increases there is clearer discrimination in favour of the photofragment H atoms which were

generated with velocities pointing along or close to the direction defined by the photolysis and probe lasers.

An example of the use of VADS is shown in Fig. 2 which compares spectra of the H atoms generated by photolysis of HI at 248 nm with and without a time delay between the photolysis and probe laser pulses. As the photofragments are both atoms, the H atoms produced are nearly monoenergetic, the only distribution of speeds resulting from formation of some of the I atoms in the $^2P_{1/2}$ excited level as well as the $^2P_{3/2}$ ground state. This splitting can be seen in the time-delayed spectrum.

With photodissociation of larger species, such as H₂S or NH₃, internal excitation in the molecular fragment is possible, just as in going from photoionization of an atom to photoionization of a molecule. The spectrum of H atoms produced from H₂S and recorded at relatively long time delays, for example, $\delta t = 210$ ns, now reveals extensive structure, indica-

ting significant production of SH in vibrational levels out to $v = 8$. This is in sharp contrast to the result for NH₃. Here, the Doppler profile of the H-atom line is essentially unstructured and undergoes little change as the pulse-probe delay is increased. This result demonstrates extensive internal excitation of the NH₃.

Wittig and co-workers' latest paper places VADS spectroscopy on a firm quantitative footing. Already the technique has provided valuable information about product state distributions, scattering anisotropies and bond dissociation energies. An exciting prospect is its application to individual states of molecular photofragments so that one could examine how distributions of one molecular photofragment are correlated with particular states of the second molecular photofragment. □

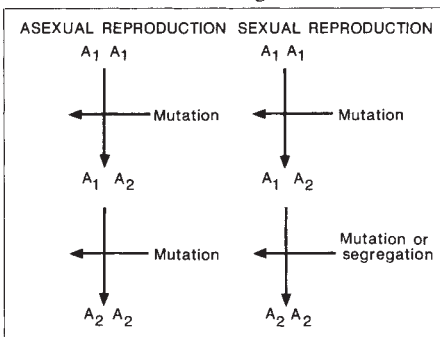
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EVOLUTIONARY BIOLOGY

A new reason for having sex

James J. Bull and Paul H. Harvey

IN A sexual population, genes from different individuals can come together in a single descendant. This construction of new genotypes from pre-existing variability involves segregation and recombination. In segregation, homologous chromosomes separate during meiosis to yield haploid gametes. Recombination describes the fact that an individual's gametes contain a



different assortment of alleles from those found in either of the gametes from which it was derived. Like segregation, recombination occurs during meiosis, but it involves crossing over between homologous chromosomes as well as the independent assortment of maternal and paternal homologues. Theories for the evolution of sex have typically focused on the advantages of the combined effects of recombination and segregation¹. On page 300 of this issue², Kirkpatrick and Jenkins describe a potentially powerful effect of segregation alone on the maintenance of sexual reproduction.

Both R. A. Fisher³ and H. J. Muller⁴ pointed out that, in a sexual population,

advantageous mutations arising at two different loci in two parents can be combined in one individual in a later generation. In asexual populations, advantageous mutations arising in separate individuals cannot be brought into the same descendant. The segregation advantage, which applies even to a single locus in a diploid species, is deceptively similar. Consider a population containing individuals of genotype A₁A₁. An advantageous mutation arises, creating genotype A₁A₂. In the absence of segregation, the mutation A₂ is destined to remain in heterozygous condition until, at some time in the future, a second mutation occurs at the homologous locus in a descendant to create A₂A₂. With segregation, however, no further mutations are necessary to yield the homozygote (see figure).

When is the single-mutation segregational advantage to sexual reproduction likely to be more important than the double-mutation recombination advantage? The recombination model provides virtually no advantage for sex in a small population because, as two beneficial mutations are unlikely to arise at the same time, the first one will be fixed in all individuals before the second one appears. With the advantage of segregation, however, a sexual population produces homozygotes within a few generations, whereas an asexual population — with heterozygotes only for the advantageous allele — must await a second beneficial mutation at that locus. By compounding this process over several non-recombining loci in a genome, Kirkpatrick and Jenkins demon-

ALLOSTERIC PROTEINS

Confirmations and limitations

Joël Janin

trate how huge advantages can apply to sex², which more than compensate for the famous twofold cost⁵.

The segregation advantage is appealing because it applies under biologically realistic conditions: population sizes can be small as well as large; an appreciable proportion of the life cycle is spent as a diploid; and beneficial mutations are recessive or partly dominant. On the other hand, the model's efficacy in maintaining sex is mitigated if heterozygote superiority or complete dominance of beneficial mutations are commonplace, or if much of the life cycle is haploid. In the latter case, an advantage of segregation applies only to the diploid phase and will be tempered by overlap in gene expression between the haploid and diploid phases. Segregation alone probably cannot explain the maintenance of sex in bryophytes, for example. Furthermore, although the model does provide an advantage of sexual relative to asexual reproduction, it does not explain why self-fertilization (such as with polar-body fusion) would be at a disadvantage. Indeed, the very advantage of segregation proposed by Kirkpatrick and Jenkins would be maximized under self-fertilization, yet disadvantages of self-fertilization are not in doubt (deleterious mutations are rendered homozygous).

Maynard Smith, in his book dedicated to the evolution and maintenance of sexual reproduction, wondered whether some piece of the jigsaw of our understanding of sex is lacking⁶. Kirkpatrick and Jenkins have discovered such a piece. Their new model can be added to a handful of others that invoke an advantage of recombination⁷. Unfortunately, the accumulation of theories has not been accompanied by a corresponding growth of empirical work addressing the different mechanisms underlying these models⁸. The field, in fact, is one in which ideas have perished or persisted largely on the basis of plausibility arguments rather than on facts. The time is ripe for empiricists, using yeast or other short-generation organisms, to generate the data that will test the theoretical developments. □

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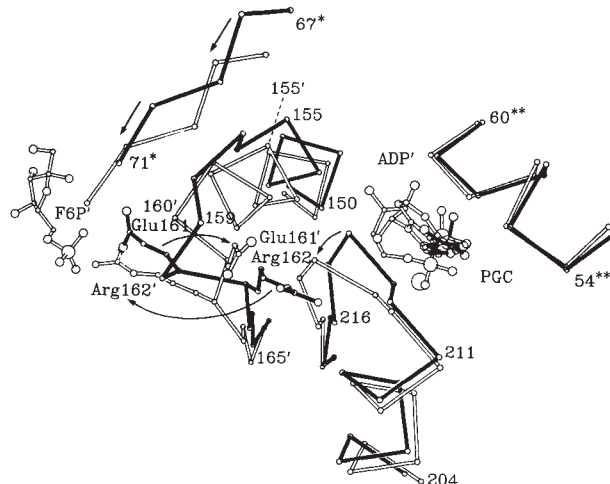
ALLOSTERY, first introduced¹ conceptually in 1963 and made famous two years later in a celebrated paper² by Monod, Wyman and Changeux, has lost none of its appeal today. Biochemists, molecular biologists and X-ray crystallographers continue to be fascinated by allosteric proteins, which can shift reversibly between different stable conformations, and whose regulatory properties are essential to biological control. At a recent conference^{*}, it emerged that, in many cases, allosteric regulation can now be interpreted at the atomic level.

Allostery sprang from the conviction

crystals of the T form of haemoglobin, the tertiary changes at oxygenation are restricted (G. Dodson, University of York).

Similar coupling of changes in structure and affinity occurs in *Escherichia coli* aspartate transcarbamylase (ATCase)³, which catalyses the first step of pyrimidine biosynthesis. Its reaction with its substrate aspartate is cooperative, and is inhibited by feedback from the end-product cytosine triphosphate (CTP). When binding with substrate or its analogues, the molecule elongates by 12 Å as its subunits rotate. Domain movements within the subunits affect the geometry of the active sites,

A structural basis for allosteric control of bacterial phosphofructokinases. The substrate site for fructose-6-phosphate (F6P) on one subunit is separated from the allosteric site by helix 150–160. In the R form (open bonds), the allosteric site is occupied by the activator ADP; in the T form (solid bonds), by the inhibitor PGC. Note that the site is located at the subunit interface, as residues 54**–60** belong to an adjacent subunit. In the T form, the last turn of the helix (residues 156–160) unwinds. Arg 162, which interacts with the phosphate group on F6P in the R form, is replaced by Glu 161, creating a repulsive charge interaction. (Courtesy of P. Evans.)



that mammalian oxygen transport and bacterial biosynthetic pathways regulated by feedback have a common mechanism. At the meeting, J. P. Changeux (Institute Pasteur, Paris) recalled how the principles of the model of Monod *et al.* were developed: allosteric sites are regulatory binding sites distinct from active sites, protein molecules have more than one state, each with its shape and function, and subunits assemble into symmetrical structures. The structural basis for the theory was provided by the X-ray analysis of the R and T forms of haemoglobin, the history of which was told by M. Perutz (Laboratory of Molecular Biology, Cambridge).

In haemoglobin, changes of quaternary structure involve rotations of subunits accompanied by smaller atomic displacements within them (tertiary structure changes) as well as alterations of the haem environment. The changes determine the affinity of the molecule for oxygen, and the effects are tightly coupled: when subunit movements are prevented, as in

showing that cooperativity has the same origin as in haemoglobin (W. N. Lipcomb, Harvard University).

By contrast, the functioning of allosteric effectors in ATCase is still poorly understood. The inhibitor CTP and the activator ATP bind far from the active sites, so that their influence must extend through several subunit or domain interfaces. But a powerful combination of genetic, biochemical and structural approaches by R. Kantowitz (Boston College), R. Cunin (Free University, Brussels) and G. Herve (CNRS, Gif-sur-Yvette) promises soon to unravel this mystery of signal communication.

Meanwhile, there is exciting news of the allosteric mechanism of muscle glycogen phosphorylase and of bacterial phosphofructokinases from L. Johnson (University of Oxford) and P. Evans (Laboratory of Molecular Biology, Cambridge). The structure of the low-activity or T form of glycogen phosphorylase⁴ has been known for several years for both the *a* and *b* forms, the first of which is a phosphorylated derivative of the second.

The high-activity R structure has just

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*The Jacques Monod Conference *Twenty-five years of allostery* was organized by CNRS and held on 28–31 March 1989, at Roscoff, Brittany, France.

been solved. It shows quaternary changes, subunit rotations again, and tertiary changes similar to those seen between the *a* and *b* forms. Signal transmission results from a change of subunit packing and alterations of the tertiary structure of the α -helix linking the subunit interface and the catalytic site. In the *a* form, the sequence around the phosphorylated serine residue has α -helical form, forming new interactions between subunits in the dimer. The effector AMP site is located near the dimer interface, far from the pyridoxal phosphate moiety at the active site.

Changes in substrate affinity are best understood in bacterial phosphofructokinase³, a tetrameric enzyme in which effectors bind close to subunit interfaces and to the substrate site of a neighbouring subunit (see figure). The differences between the two allosteric forms include a remarkable local change: the end of an α -helix situated between the effector and substrate sites unfolds, and an arginine side chain is replaced by glutamic acid. Because, in the R form, the arginine interacts with the phosphate group of the substrate fructose 6-phosphate, the affinity for the substrate falls dramatically. Classical kinetic studies of *E. coli* phosphofructokinase implying a very large change of affinity between the R and T forms are nicely vindicated by this new structure.

The activity of mammalian phosphofructokinases is regulated by association-dissociation rather than by conformation changes alone (J. Lee, University of Missouri, St Louis). This mechanism is easily reconciled with allostery. In haemoglobin, subunit dissociation is also coupled to substrate binding, although this has no physiological function in mammals. Careful measurements of the dissociation constant yield values of the relative free energy of ligated forms with between one and four ligands. The results suggest that molecules with two bound ligands may represent a third allosteric form (G. Ackers, Johns Hopkins University), possibly in agreement with direct measurements of the relative abundance of the various ligated forms by cryoscopic methods (M. Perella, University of Milan).

While cooperativity can be accommodated in the original model², anti-cooperativity cannot be, but is instead the basis of the model of Koshland *et al.*⁶. Its structural interpretation has now emerged from the X-ray study of glyceraldehyde 3-phosphate dehydrogenase from *Bacillus stearothermophilus*⁷. High-resolution structures for the tetramer and for several complexes with the coenzyme NAD were described by A. Wonacott (Imperial College, London). In the free enzyme, the subunits are identical and have dihedral symmetry. In a newly determined structure with one NAD per tetramer, the symmetry is lost because of alterations of tertiary structure induced by ligation.

Further changes of the same nature cannot easily be accommodated in other subunits unless the quaternary structure also changes, which it does in the fully ligated protein, restoring dihedral symmetry.

While the interactions of bacterial repressor proteins with DNA may not be part of allostery in the strict sense, they certainly belong to a Jacques Monod conference. Examples were presented of the *trp* repressor⁸ (P. Sigler, University of Chicago) and the *met* repressor (S. Phillips, University of Leeds). Though unrelated, both proteins are dimers, they carry the characteristic helix-turn-helix motif for DNA binding and the co-repressor site is near the interface with DNA. The co-repressor, tryptophan in one case and S-adenosyl-methionine in the other, appears to make direct contacts to phosphate groups on DNA. Its binding induces conformation changes in *trp*, but not in *met*.

The possibility of direct interaction between ligands as an alternative to allostery was raised¹ in 1963, and may be the basis for the non-covalent regulation of another classical regulatory enzyme, *E. coli* glutamine synthetase. X-ray analysis reveals that tryptophan and histidine, two of a score of nitrogen metabolites among its inhibitors, bind close to the active site and may overlap with the substrate (D. Eisenberg, University of California, Los Angeles). But covalent regulation is more efficient than non-covalent inhibition (E. Stadtman, National Institutes of Health) and the dramatic effects of adenylation of tyrosine residues far from active sites, still to be explained at the atomic level, are not unlike those induced by effectors in allosteric enzymes.

While the scope of allostery may be less than its original authors hoped, structural data on haemoglobin and several allosteric enzymes fit the theory remarkably well, given that so little was known in 1965. Effector sites, subunit movements and symmetry are consistently observed, and their thermodynamic coupling explains cooperativity and regulation. Equilibrium binding studies and kinetics may be more difficult to reconcile with the simplicity of the equations of the model of Monod *et al.*, yet it remains the best we have — if only for its cartesian elegance. □

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Sky snatcher

TELEVISION is a massive time-waster. One can skim rapidly through a book, a journal, or a newspaper to see if anything appeals; but television demands real-time attention. It seldom repays it. Even a dedicated channel hopper can waste hours every day in a tedious hunt for worthwhile snippets of entertainment. Now Daedalus automates that hunt.

DREADCO's 'Snatcher' is a combined video recorder and pattern-recognition computer. It receives all channels simultaneously, and records any topic or type of image that it has been programmed to watch out for. At daily or weekly intervals, its owner can play back the tape: a surrealistic succession of clips and snatches encapsulating everything he would have found interesting for the whole period. All the boredom of trawling the air waves in real time is avoided.

The main problem is how to tell the Snatcher what to watch for. The audio channel is the simplest indicator; the telephone-tapping industry has already developed very effective computer algorithms for identifying specific voices or words in context. Those who want every utterance of Mrs Thatcher, or every reference to reinforced concrete, will be quite easy to satisfy. But a visual interest, perhaps in horses or figure-skating or bedroom scenes, will test current pattern-recognition techniques to the limit. The viewer will have to provide a coded set of visual cues or patterns for the Snatcher to watch for, and optimize them until its 'catch' is satisfactory.

For those households which keep the TV switched on all the time, but look at it only occasionally, Daedalus has a more fundamental approach. He plans to equip the Snatcher with a viewing sensor. It fits on the TV set, and scans the room with an infrared beam, seeking the characteristic front-surface reflection returned by eyes looking at the set. An 'artificial intelligence' learning algorithm will thus be able to discover which types of scene are actually watched. Over the weeks it will refine a complex, inscrutable, but increasingly reliable criterion of viewability. Soon the Snatcher will be able to watch on its own, recording everything of household interest.

Vast amounts of time will thus be reclaimed for reality. Most TV is really watched negatively — thus we watch the news to reassure ourselves that nothing very important has happened. The Snatcher will extend that assurance to the whole broadcast output. Pressure groups like anti-pornographers will also welcome the Snatcher. They could program it to detect every example of risqué viewing, and to send off a computer-generated letter of complaint. No human viewer need risk being corrupted.

David Jones

New 3-set Venn diagram

SIR—Recently, one of us (A.W.F. Edwards *New Scient.* 121, 51–56; 1989) gave a solution to the problem of constructing a Venn diagram for an arbitrary number of sets (Fig. 1a) and commented

to be different to the usual form (Fig. 2).

It is easy to prove that there are no further forms for three sets, not counting as distinct any obtained by set boundaries merely touching without crossing. The

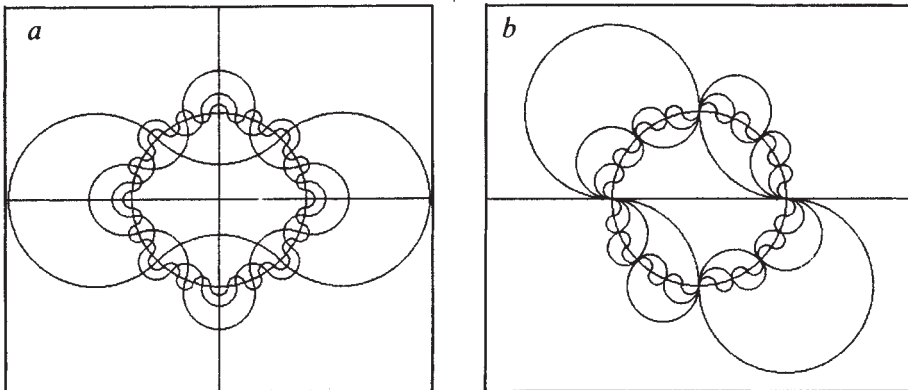


FIG. 1 a, The Edwards map of a 7-set Venn diagram. The boundaries of the first two sets are the straight lines; subsequent sets are concentric 'cogwheels' with 2, 4, 8, ... teeth, until the last set, which is the circle. b, An alternative version for six sets obtained by rotating the sets of the 7-set Edwards map. In the process the first two sets coincide, reducing the total number by one.

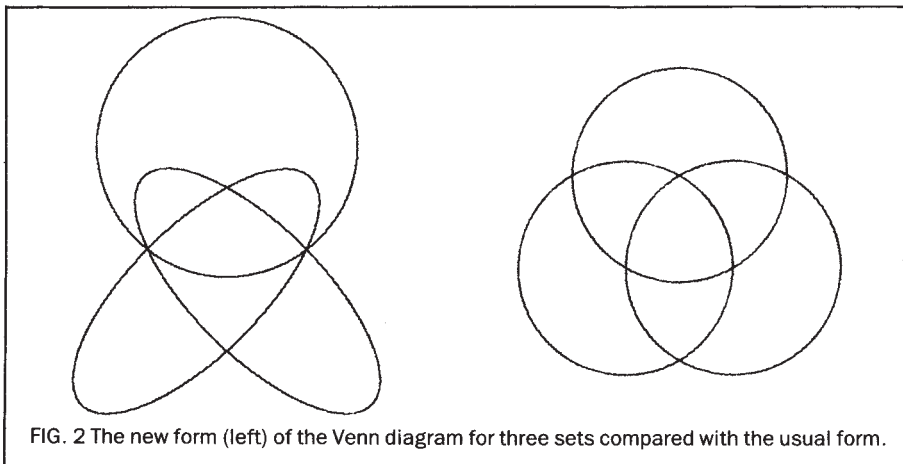


FIG. 2 The new form (left) of the Venn diagram for three sets compared with the usual form.

on the connection it revealed between Venn diagrams and the Gray code of communication theory. This prompted the other to remark that an equivalent representation of such an 'Edwards map' is the family of cosine curves $y = (\cos 2^n x)/2^n$, $0 \leq x \leq \pi$, $n = 0, 1, 2, \dots$, and that the family of sine curves $y = (\sin 2^n x)/2^n$, $0 \leq x \leq 2\pi$, $n = 0, 1, 2, \dots$, is an alternative which in turn reveals a similar connection with ordinary binary code. Although the association of these functions with the Gray and binary codes is not new, we believe their association with Venn diagrams is.

Because the sine curves are but phase-shifted cosine curves, the analogous version of the Edwards map is obtained by rotating each set of the map through an angle corresponding to one quarter of a wavelength (Fig. 1b). (A model made with sets copied onto transparent sheets and concentrically mounted is a useful aid.) When this is done, the topological form of the diagram for three sets is found

number of forms for k sets ($k > 3$) is still an open question.

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Pulsar structure

SIR—The observed^{1,2} properties of PSR0535–69 (the SN1987A pulsar) may be explained if the neutron star is internally convecting or stirred. I reject the apparently natural interpretation of the 8-hour period as an orbital period because the projected semimajor axis would be smaller than the progenitor star; it is not possible for a Jupiter-like companion (implied by the small mass function) to survive deep inside another star, and matter from a stellar interior has too much entropy to condense into such an object.

I suggest that the roughly sinusoidal variations may be timing noise resembling that observed (at lower amplitude) in

many older pulsars. This hypothesis naturally explains the discrepancies between the pure sinusoid and the data, and predicts that the period variations will not remain sinusoidal.

Alternatively, the sinusoidal part of the period variations may be attributed to torsional magnetohydrodynamic oscillations of the neutron star's crust with respect to its interior, or to Tkachenko modes³. Magnetohydrodynamic oscillations would be expected to have periods of the order of $r(8\pi\varrho_{\text{eff}}/B^2)^{1/2}$, where r is the radius, ϱ_{eff} is the density and B is the magnetic field of the neutron star. Adopting $\varrho_{\text{eff}} \approx 10^{14} \text{ g cm}^{-3}$ and $r = 10 \text{ km}$ yields $B \approx 2 \times 10^9 \text{ gauss}$. Even if oscillations are present the non-sinusoidal part of the residuals implies the existence of timing noise.

Independent estimates of the magnetic field are consistent with the low values required by the torsional oscillation model. First, the reported bound on the period derivative implies an effective magnetic moment $\mu < 10^{29} I_{45}^{1/2} \text{ G cm}^3$, where I_{45} is the moment of inertia of the neutron star in units of 10^{45} g cm^2 . Second, the optical light curve of SN1987A suggests⁴ an estimate of $\sim 10^{38} \text{ erg s}$ for the pulsar contribution, implying $\mu \approx 4 \times 10^{26} \varepsilon^{-1/2} \text{ G cm}^3$, where ε is the efficiency of converting spin-down power to visible radiation. Unless ε is extremely small ($< 10^{-7}$), μ is less than typical pulsar values. I conclude that PSR0535–69 was born spinning rapidly and with a low magnetic field, thus obviating the need⁵ for accretional spin up in a binary system to produce such an object.

If the surface layers of the neutron star are undergoing convection then both the pulse intensity variations (and disappearance) and the timing noise may be explained. Magnetic flux is frozen on the required timescale (hours), so subduction and upwelling of surface material will change the exterior field configuration, and hence will alter the pulse power and angular distribution. Surface convection will also produce fluctuations in I , explaining the observed timing noise. If the surface is solid⁶, strains will be imposed and relax and 'mountains' will rise and fall, in a similar manner to terrestrial plate tectonics. Even liquid matter will not be exactly barytropic because hysteresis in achieving β -equilibrium (a source of substantial bulk viscosity) means that convection could also lead to fluctuations in I . A number of forces may drive convection or stir the neutron star — cooling of upper layers by neutrino bremsstrahlung⁶, Eddington–Sweet currents resulting from variation of cooling rates with latitude (pronounced in such a rapidly rotating object), possible internal differential rotation and so on.

Fluctuations in I of magnitude $\sim 10^{-6} I$ are inferred from the spin rate. If the

moment-of-inertia tensor of PSR0535-69 contains non-axisymmetric components ΔI comparable to the irregular fluctuations in I , then the rate of gravitational quadrupole radiation is $\sim 10^{43} (\Delta I_{39})^2 \text{ erg s}$. (Here $I_{39} = I/10^{39} \text{ g cm}^2$.) The corresponding metric fluctuation at the Earth is $h \approx 10^{-25} \Delta I_{39}$. The empirical bound on the period derivative imposes an upper bound of $5 \times 10^{43} I_{45} \text{ erg s}$ on the radiated power, unless it is offset by another process. The quadrupole power is radiated at the accurately known frequency 2ν . Higher non-axisymmetric multipole moments radiate at frequencies 3ν , 4ν and so on.

The rapid spin of PSR0535-69, approaching the limiting angular velocity of neutron stars⁷, naturally suggests that it was spun up to this limit when it was formed, and is now slowing by the emission of electromagnetic or gravitational radiation. Internal cooling by neutrino emission produces contraction and angular momentum-conserving spin up. Exchange of angular momentum with a differentially rotating core may lead to a period derivative with either sign, or to fluctuations. Differential internal rotation may heat the star. It is possible that the neutron star is surrounded by an accretion

disk of matter left over from the supernova with excess angular momentum. Then, as angular momentum is radiated away, accretion will maintain the pulsar on the $\nu_{\text{max}}(M_{\text{NS}})$ relation as M_{NS} , the neutron-star mass, increases. As in accreting binary neutron stars, irregularities in angular-momentum transfer could produce irregular variations in ν of the type observed. Accretion suggests the exciting possibility that the pulsar may be pushed over its limiting mass and collapse to a black hole or a more exotic configuration; the timescale would be determined by the (unknown) angular-momentum loss rate, and could be short.

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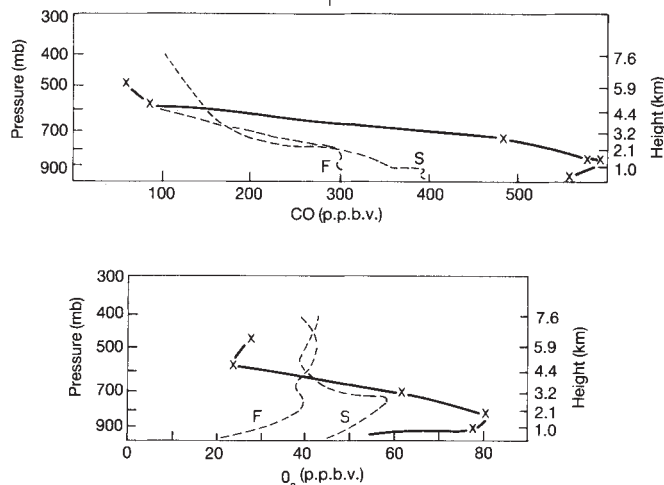
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O₃ and CO from burning sugar cane

SIR—Brazil produced the first alcohol-powered automobiles after 1980, following a National Plan for reducing oil imports. They proved to be a great success: today 99 per cent of all manufactured vehicles for the internal market run on alcohol. Tropical countries make their sugar and alcohol from sugar cane. The manual harvest of sugar cane requires the initial burning of its foliage. This burning increased sharply in Brazil after the National Alcohol Programme was started. In 1988, more than four million hectares of sugar cane were grown in Brazil, about half of which was in the state of São Paulo.

To investigate the effect of such large-

scale burning on the lower atmosphere away from the sources, two ozone sensors were installed on an airplane. One sensor used the ultraviolet absorption technique of ozone detection, the other used the chemiluminescent reaction between ozone and ethylene. Practically the same concentrations of ozone were detected by each instrument. In addition, air samples were collected systematically in special stainless-steel internally-electropolished cans (developed for sampling purposes by R. Rasmussen, Oregon Graduate Center) for subsequent CO, CH₄ and CO₂ analysis. Details of the analysis techniques are described elsewhere^{1,2}.



Atmospheric concentrations of CO and O₃ as a result of sugar cane burning (solid line) compared with biomass burning (broken lines).

The figure shows the profiles obtained for O₃ and CO. A very concentrated layer of gases was present, with peak concentrations at about two kilometres of 80 parts per 10⁹ volume (p.p.b.v.) O₃ and 580 p.p.b.v. CO. These high concentrations, obtained in the rural area of the western part of the state of São Paulo, are about twice those reported for biomass burning events in Amazonia³ over forest areas and savannah, and shown in the figure by dashed lines F and S, respectively. High concentrations of CH₄ (1,756 p.p.b.v.) and CO₂ (409 p.p.m.v.) were present at 1,500 metres. During the period of the experiment, the average diurnal variations in ground-based O₃ at two rural sites were practically identical to the typical urban variation observed at São José dos Campos, with daytime ozone values between 45 and 60 p.p.b.v.

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Chaotic names

SIR—Nobody would defend the present state of the genetic nomenclature of *Drosophila*. It is a mess. This is no excuse, however, for the cavalier attitude of some workers in the field. We refer, in this instance (but there are many others), to the paper by S.M. Cohen *et al.* (*Nature* **338**, 432-434; 1989) on the characterization of a gene they call *Distal-less*. As they acknowledge, this gene had been described two years previously by Sunkel and Whittle, who called it *Brista*—a perfectly good name and the one that should be used, as it has priority. If everyone was to follow the example of Cohen *et al.* a mess would rapidly become chaos.

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Erratum

In the Scientific Correspondence on cold fusion in the 18 May issue of *Nature* (p.184), the 24th line from the bottom of the 2nd column should read: "This value is a factor of 50 times higher than the rate that would be calculated on the basis of the results in Fig. 2 . . .". On the same page the first line of the acknowledgements should read: "We thank C. Kurz . . .".

Decline of European forests

SIR—Forests are more than trees. In their Commentary article on the status of European forests, Blank *et al.*¹ based their account on the results of national surveys of the health of tree crowns. Their discussion of forest decline is mainly from a population perspective, which does not give due weight to the soil aspects of the problem. We suggest that an ecosystem approach provides a more complete picture.

Measurement of input-output budgets for the principal ions is a prerequisite for a classification of the status of a forest as an ecosystem. In 1979, Ulrich *et al.*² showed that, in central Europe, acid deposition can be the main driving force in these budgets. At Solling and Lange Bramke (FRG), two sites for which ecosystem budgets are available from before and after the start of forest decline, acid deposition has caused changes in the pools of major ions in the soil. Among these changes are decreasing storage of magnesium and increasing storage of nitrogen^{3,4}. Such budgets have now been measured at over 100 sites, and the results are alarming⁵.

One of the advantages of monitoring ion fluxes is that throughput measurements give a cheap and reliable indicator of the total atmospheric input of sulphate⁶. In Europe the fluxes of hydrogen ion and sulphate beneath forest canopies closely match the large-scale distribution of concentrations of sulphur dioxide⁵. They follow the same north-south gradients, with a maximum belt across Britain, the Netherlands, West Germany, Czechoslovakia, East Germany, Poland and the Soviet Union. In these areas the output fluxes in seepage water are dominated by sulphate and nitrate; in other areas that are not subject to acid deposition, chloride, bicarbonate and organic anions dominate.

In Europe there are large regional gradients in atmospheric loadings and in soil sensitivity to acidification. We can reasonably assume that almost all soils in the high-deposition area have undergone long-term, gradual changes in their nutrient status. In that case there will have been two possible responses—first, that essential nutrients such as magnesium became limiting, especially as the previously limiting nutrient nitrogen was increasing; second, that potentially toxic ions such as aluminium, manganese and hydrogen increased when calcium and magnesium decreased, with adverse consequences for the functioning of the fine root system^{3,4}. That is why Ulrich *et al.*² predicted large-scale forest decline in central Europe as long ago as the mid-1970s.

We think that two factors led to the increasing awareness of forest decline

after 1982: the appearance of completely new symptoms (at least regionally, the occurrence of yellowing), and a parallel occurrence of other unspecific symptoms (crown thinning). The surveys of needle mass in the following years brought all the different symptoms into a one-dimensional damage classification for each site, resulting in confusion about the possible causes⁷. For Norway spruce alone there are at least three (possibly five) different types of damage⁸.

Some of these above-ground mainly unspecific symptoms are probably a consequence of short-term climatic fluctuations over periods of up to seven years. Yet such short-term effects may still be influenced by the acid-base status of the soil, as exemplified by the response of nitrate⁹. The fact that these changes include periods of recovery, as pointed out by Blank *et al.*¹, does not mean that gradual deterioration of central European forests is not occurring.

Perhaps the clearest example comes from the Harz mountains, where for the first time regional damage inventories by infrared aerial photography were carried out separately for the two main symptom types¹⁰. The distribution of both types, yellowing (type 1) and loss of green or brown needles (type 2) are clearly related to soil sensitivity (and deposition) factors^{4,10}. If both types are pooled into a total damage figure for Norway spruce, all correspondence with the possible stress factors discussed by Blank *et al.*¹ is lost. A hypothesis based on soil acidification as the key predisposing factor described such differences in damage symptoms locally for the Bramke Valley, as well as regionally for the Harz¹ and for central Europe².

As in the early 1980s, indirect effects of acid deposition on the acid-base status of forest soils have emerged as a major factor in understanding the status of forest ecosystems in Europe. The implications for forest management and air-pollution control policy are considerable; the increasing acceptance of the concept of

critical load in controlling sulphur and nitrogen emissions is a step in the right direction. Many of the causal links to explain short-term variations in tree health, or the role of direct effects (for example by ozone), are still missing. But the points made above show that there is a view of forest decline rather different to the one expressed by Blank and his colleagues.

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BLANK *ET AL.* REPLY—Although Hauhs and Ulrich say they provide a different view of forest decline to that which we presented in our Commentary article, we note that there are now many areas of agreement. There have been considerable changes from the earlier arguments put forward by Ulrich and colleagues¹, the most important of which is the acceptance of regional decline types and the importance of short-term climatic fluctuations in damage development. We are in agreement with Ulrich and colleagues that the forest damage surveys have severe limitations, and that the results cannot be used to quantify the role of some factors (for example air pollution). We also agree that forest decline can no longer be seen as a one-way street, but that there has been recovery, indicating reversibility.

The area of continuing disagreement revolves around whether the current recovery is a transient phenomenon in a continuing decline, or is a clear sign of a reverse. The concept of continuing forest decline depends upon the argument that soil acidification is the long-term driving force across Europe. This view ignores the existence of regional decline types with distinct symptoms and combinations of causal factors. As we pointed out in our article, and as Hauhs and Ulrich agree, there are at least three, probably five, types of Norway spruce decline. We know now that at least one of these types is not related to soil acidification (type 3 decline in the foothills of the Alps, caused by needle-cast fungi²).

The main step forward has been in understanding the causes of type 1 spruce decline (needle yellowing in the German *Mittelgebirge*; *montane Vergilbung*²). We know that the symptoms result from magnesium deficiency³. The soils in the areas concerned have always been marginal for magnesium because of its slow release by weathering and low atmospheric input. Depletion of exchangeable magnesium has been accelerated by planting Norway spruce monocultures and by increased leaching resulting from relatively high inputs of mobile anions. Increased soil

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acidity could reduce magnesium uptake by lowering the magnesium/aluminium ratio in soil solution.

Unlike Hauhs and Ulrich, we believe that aluminium toxicity is unlikely to occur at most sites. It is now known that the Solling is an exceptional area regarding aluminium availability⁴. Part of the increase in magnesium deficiency between 1976 and 1988 was due to the drought years of 1975–76 and 1981–84. In dry years there may be less magnesium released from litter, less magnesium uptake from subsoil, poorer root development and more leaching due to nitrification. Ozone may be involved in reducing root growth in dry years, but the effect on foliar leaching is small. In wetter years, litter mineralization and soil weathering should supply more magnesium and the symptoms will be reduced. This explains the reversibility of type 1 decline in West Germany since 1985.

An important factor in magnesium depletion in sensitive areas is deposition of sulphur and nitrogen. However, Hauhs and Ulrich fail to acknowledge that tree harvesting is an equally important mechanism of removing magnesium, as demonstrated in element budget studies of the type advocated by Hauhs⁵. The obvious immediate remedial action from our analysis of the problem should be based on the addition of magnesium to soils. Adding lime or limestone, as suggested by Ulrich⁵, will not be effective in correcting the symptoms of magnesium deficiency.

In a European context, it is relevant that magnesium deficiency has not been apparent in the forests of countries along the maritime seaboard of northern Europe (the reason is that the rate of magnesium deposition in precipitation is much greater there than in central Europe). Consequently, the symptoms of magnesium deficiency have not been seen in Britain or Norway, even in areas of high acid deposition, nor do coniferous forests in these regions respond to fertilization with magnesium.

Research on other types of forest damage has not advanced as far as that on type 1 spruce decline. The practical implications of understanding these scientific issues are profound. Soil acidification can be reversed by liming; nutrient limitations require re-addition of the limiting nutrients; direct effects of pollution require reduction of the pollutants in question; pathogen infections require symptomatic treatment. But if the causes

are not properly identified then resources will be wasted in ineffective treatments.

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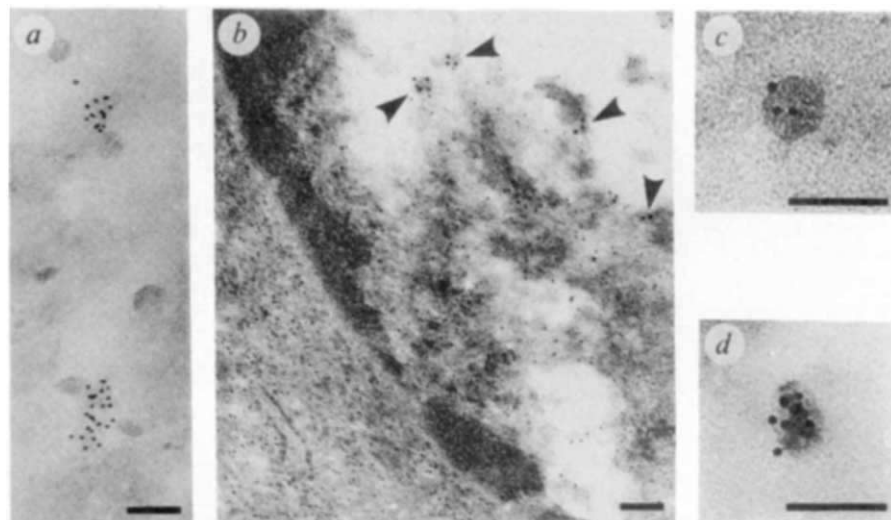
Baikal seal virus

SIR—We have recently reported^{1,2} evidence that the cause of the disease in Lake Baikal seals (*Phoca siberica*) in the autumn of

studied for comparison.

Gold particles in livers of both seal and dog were present mainly as clusters, which were particularly abundant in cell nuclei (*a, b* in the figure). Some clusters reside on virus-like particles of oval or hexagonal form having a diameter of about 80 nm. (*c, d* in the figure). Similar patterns were seen in kidneys, but in spleens gold spheres were present as single particles rather than clusters. The proportion of cells containing morbillivirus antigens was high, indicating the severity of infection. Practically no gold particles were found in controls treated with gold-protein A in the absence of monoclonal antibodies.

The similarity of the patterns obtained



Immunoelectron microscopy of dog liver (*a, c*) and seal liver (*b, d*). Scale bar, 100 nm. Tissues were fixed in 4% CHO/0.4% glutaric aldehyde phosphate for 2 h, kept in 2.1 M sucrose for 1 h. Sections (15–20 μ m) were cut at -10°C into 10 mM Tris-HCl (pH 8), 0.15 M NaCl, 0.05% Tween-20, 0.1% ovalbumin. Monoclonal antibodies BK-228 (raised in mice against human measles virus strain Leningrad-16) and LEC-27 (raised against measles virus from a patient with sclerosing panencephalitis) were applied at concentrations 0.135 or 1.35 $\mu\text{g/ml}$ (BK-228), and 0.16 or 1.6 $\mu\text{g ml}^{-1}$ (LEC-27). Incubation overnight at 4°C was followed by addition of colloidal gold-protein A (Bios, Novosibirsk; 15 nm particles). After 1 h, sections were dehydrated and embedded in Epon-Araldite. Ultra-thin sections were cut, contrasted with uranyl acetate, and examined with a Philips EM-410 electron microscope at 60 kV.

1987 was infection by a morbillivirus similar to canine distemper virus (CDV). The same, or a very similar, virus attacked European seals the following spring^{3–7}. By means of electron microscopy combined with immunogold staining, we can now confirm the presence of morbillivirus antigens in the tissues of a seal.

The seal, kept in captivity, died with typical symptoms of distemper. Its serum contained anti/CDV antibodies, and its spleen and liver gave a positive reaction with oligonucleotide probes¹. Pieces of liver, kidney and spleen were fixed, sectioned, exposed first to monoclonal antibodies against the morbillivirus that causes measles, then to colloidal gold-protein A. Tissues of a dog infected with CDV were

with seal and dog tissues is in favour of the suggestion that a morbillivirus similar to CDV caused the disease of Baikal seals.

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Monsters on the loose

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Anti-Evolution: An Annotated Bibliography. By Tom McIver. McFarland, Box 611, Jefferson, North Carolina 28640: 1989. Pp. 385. \$39.95. In Britain distributed by Bailey Bros & Swinfen, Warner House, Folkestone, Kent CT19 6PH, £29.95.

BESTIARIES are wonderful things. Some are books for children, filled with pictures of improbable monsters, dinosaurs and the like, that crouch under beds or live grouchy in trash cans. But just occasionally the creatures escape: imagined amusement becomes belief and the result is a tearful and distraught child. Science too has its bestiaries, collections of discarded beliefs. On occasion they too get loose, especially the ones about dinosaurs, and cause damage. If there is a monster about, it is best to know what it looks like. Tom McIver has provided us with a splendid bestiary of anti-evolution ideas. The book is nothing more than a catalogue of abstracts, a stamp collection, yet it is a fascinating work, and well worth its cost, either for a chuckle or, on those unpleasant occasions, to face up to a nightmare on the rampage.

McIver has covered his subject very widely. The book is without narrative, and is simply a collection of paragraph-long summaries of works against evolution or in favour of 'creationism'. These fall broadly into two categories: those that date from before Darwin, and the newer, twentieth-century attacks on evolution. Interestingly, there are links between the two traditions. Some of these links are respectable, such as those running through Sir William Dawson or Sir Richard Owen. Others are more repellent: for example, if McIver is correct, there is a line of descent from Cuvier through Agassiz to modern revisionist historians. Not all of the works listed stem from Protestant Christianity. Some are Roman Catholic, others are based on Islam or on Indian sources, yet others range from the outpourings of neo-Nazis to quantum mystics: anti-evolution is a worldwide phenomenon. Some of the work listed is hilarious, a little (including *Mein Kampf*) is an awful reality.

The bulk of the literature against evolution is based as much on the sands of poor theology as on scientific ignorance. Martin Luther once observed that the curses of the godless sometimes sound better in God's ear than the hallelujahs of the pious. To deny scientific truth may seem pious to some, but sometimes such denials deserve what they receive. I am reminded of a story in my own family, of the discovery of a skull of a pareiasaur, the 'Blinkwater Monster', a century and a half ago in South Africa. As the intrepid geologist chipped the skull out of the rock, a young farmer rode up. The farmer was

very puzzled. "Do you ever read your Bible?" said the geologist. "Oh yes", was the response. "Well", continued the geologist, "did you never read that, when Noah was in the ark, one of the pair of wildebeest jumped overboard, and before Noah could get out his lifebuoy it was drowned?" The farmer believed the tale and rode off in wonder: the next day a deputation of local worthies arrived, including elders of the Kerk, to see Noah's

humour. More sadly, bigotry and fanaticism took over the remnant of creationists as the mainstream of geology left them. Perhaps most worrisome is the connection, documented in the book, between a small segment of the creationist community and those modern revisionist historians who are apologists for the Holocaust. It is a curiously illogical connection, given the popularity of social darwinism in pre-war Germany, but then illogic is close to bigotry. There is much dark insanity in the movement against evolutionary theory.

There are, of course, also many careful thinkers listed here, gold amongst dross. Virtually all good evangelical minds belong neither to nuts nor to extremists. In England, the grand old man of Liberalism, W.E. Gladstone, took time off from politics to write good calm sense about

IMAGE
UNAVAILABLE
FOR COPYRIGHT
REASONS

Faithful picture—
Noah saving the
results of the fifth
and sixth days of
creation.

Mary Evans

wildebeest. The elders, it appeared, neither knew their Bible, nor even logic (or they would have asked how the single remaining wildebeest reproduced).

Many of the more obscure works listed in McIver's bibliography are similarly entertaining. I particularly like the notion of a shell of ice orbiting the Earth, transmitting light to Earth by fibre optics. A divinely ordained solar flare caused its collapse — the Antarctic ice cap is a remnant — and sent Noah on his way (with frostbite one assumes). Then there is the comic book in which a bold anti-evolution student hero stands up to his obnoxious evolutionist professor and by bold debate reduces the professor to stammering idiocy. As an evolutionist professor myself I am well aware that the arguments of some students can reduce me to that same state, though usually not by their fluency and logic. Mixed in with the eccentrics, though, are the odder moments of greater minds. Newton, of course, is in this collection, and with his usual power. I had no idea that he had used cooling assumptions to calculate the age of the Earth as 50,000 years, pre-empting Kelvin.

Many of the early debates and some of the modern eccentrics have charm and

evolution and creation (incidentally, to recall a long-running correspondence in this journal on the 'wine-dark' sea, McIver notes that Gladstone was the author of a book on Homer's sense of colour). Perhaps the best of them all is Thomas Chalmers, author of a *Bridge-water Treatise*, scholar evangelist, worker for the poor and a founder of the Free Church of Scotland. His careful and reasoned attitude in the early 1800s still has much to teach us about integrity of belief, honesty in science and courage in conviction.

I teach a huge introductory class in Earth science, a thorough cross-section of North American humanity. Recently the creationists have become rare. More open-minded fundamentalists are common, but they are thoughtful and willing to be convinced by theological reason and scientific evidence. In contrast, there are some students who hold opinions that are much more frightening. On the lunatic fringe are the crystal worshippers, and the satanists. More disturbing is the new incarnation of eugenicists: those who believe that medicine has a duty to abort all genetic failures.

During the past two weeks I have been reading McIver's catalogue as light relief

to offset the marking of hundreds of student essays. One answer to a question on natural selection is worth quoting: "We have a curse — the emotion of compassion, which will not allow us to eliminate 'imperfect' specimens of our own". In my large sample of students this year I found no creationists but many who feel that society should use medicine to kill off all human beings who deviate from some arbitrary norm. To those who, like myself, are physically handicapped and poor performers at standard IQ tests, this is terror. Physiological prejudice is an ally of race prejudice — monsters that will not die.

Creationism, I sense, is a fading force. For that we should be thankful. As one

force fades, however, another becomes stronger. The resurgence of eugenics is an ugly challenge. Civilization is once again becoming unfashionable. In our time there are few voices like Gladstone's, able to set Midlothian and the nations afire with courageous appeals for reason, logic and humanity. In a society that no longer tolerates the halt, the lame and the meek, prejudice has found a new disguise, but it is stronger than ever. McIver's work is a valuable source book. He should now be encouraged to interpret his findings, to slaughter a few of his dragons. □

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Taking stock

Norman Myers

Floristic Inventory of Tropical Countries: The Status of Plant Systematics, Collections, and Vegetation, plus Recommendations for the Future. Edited by David G. Campbell and H. David Hammond. *The New York Botanical Garden: 1989. Pp. 545. \$89.55 (United States), \$91.40 (elsewhere).*

TROPICAL forests account for only 6 per cent of Earth's land surface, yet they harbour 125,000 or half of Earth's species of higher plants. That is, so far as we know. It is ironic that what is biotically the richest of Earth's biomes should also be the least investigated — while experiencing the most rapid rates of environmental destruction and species extinctions. Nor will we ever know how many species exist in tropical forests. They are disappearing faster than taxonomists can catalogue them, in part because of a skewed distribution of taxonomic muscle. Colombia, a country with one in eight of the world's plant species, has fewer than a dozen professional botanists, whereas Britain possesses 1,350 plant species and 1,400 botanists to look at them.

So let us welcome a book that presents a wealth of information about tropical forest floras (despite its expansive main title, the contributors confine their attention to tropical forests). There are more than 40 country analyses, all written by experts in their fields, with descriptions of forest types and distributions, vegetation maps, floristics, publications, and 'where do we stand' appraisals. Splendid stuff: substantive and methodical from start to finish, and a boon to anyone seeking a systematized collation of existing knowledge about plant stocks in tropical forests.

Curiously, however, it is the part of the book that one would expect to be strongest that turns out to be weakest. The end-

of-chapter assessments concentrate much more on the status of species inventorying than on the prospects for species survival. With the exception of certain contributions, such as that by Al Gentry on Colombia, Peru and Ecuador (which together contain almost one-fifth of all species on Earth), there is all too little, often next to nothing, on conservation. We urgently need to know much more about which forests are declining fastest, so that conservationists can plan their priorities accordingly. It is precisely field botanists, sloshing around in remote forest tracts, who can bring authoritative and first-hand reports on what is happening where.

Yet the botanist community sometimes seems reluctant to tackle the task of safeguarding the objects of its work. At the recent World Congress, at which several hundred papers on sundry aspects of the plant world were given, only a couple of dozen or so addressed conservation issues — even though there is plenty of evidence that we are surely losing one plant species per day already, and are building up to the greatest episode of botanical impoverishment in the world since the first emergence of higher plants 240 million years ago. True, some botanists, such as Peter Raven, Iain Prance, Mark Plotkin and Hugh Iltis, do not hesitate to take their cause to the larger world, notably to the political arena. But all too often they remain an honourable minority.

Symptomatic of the 'shrinking violet' problem is the delayed publication of this book. Work was declared to be largely complete three years ago. Yet the finished product gives every sign of being published in a super-scramble with, for example, many missing or inaccurate references. That is a pity, because the book is otherwise a magnificent compendium of knowledge — and lack of it. □

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Polymers in series

David R. Rosseinsky

Comprehensive Polymer Science: The Synthesis, Characterization, Reactions and Applications of Polymers. Chairman of the editorial board, Geoffrey Allen. Pergamon: 1989. Seven volumes, pp. 5,367. £1,095, \$1,995.

Encyclopedia of Polymer Science and Engineering, 2nd edn. Editor-in-chief J.I. Kroschwitz. Wiley: 1985–1990. Nineteen volumes (16 of which have been published). Subscription price \$170, £120 each volume.

THE small girl, obediently thanking grandmother for her gift of the nice book on penguins, wrote: "This book told me more about penguins than I really wanted to know". Certainly, either of these series on polymers must encompass more than even the most polymath polymerist would require, as only befits the terms *Comprehensive* and *Encyclopedia* respectively. But then, how all-pervasive (in some cases literally) are these materials, from biomedical adjuncts and prostheses, to concrete-repair additives, through every sort of mouldable or extrudable artefact.

The geneses of the two series are interesting. 'Mark', the 1960s edition of the *Encyclopedia* which appeared in alphabetical sequence of topics over several years, has been the general work of reference amidst libraries of specialist texts and series on formulations, coatings, processing, properties and the like. The second edition, incorporating two decades of innovation, was launched in 1985, with Herman Mark (at 90) still on the editorial board but with Jacqueline I. Kroschwitz as editor-in-chief. Beginning with Vol. 1, *A to Amorphous Polymers*, it was at Vol. 14, *Radiopaque Polymers to Safety*, when this review was being prepared.

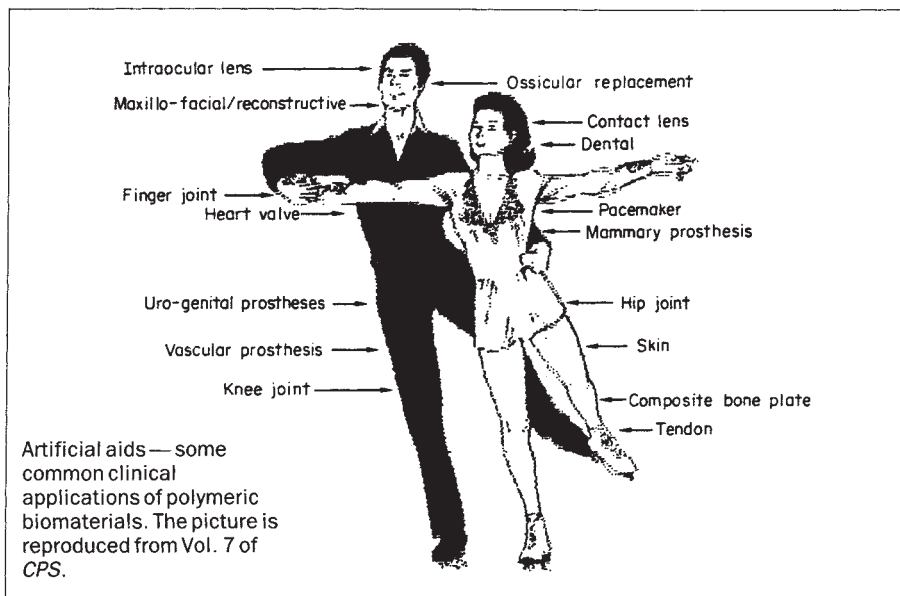
Meanwhile at Pergamon (once a literary centre in Asia Minor popularizing the use of vellum, but now situated in Oxford), general editors Sir Geoffrey Allen and Professor John Bevington were engendering the Athena-like parturition, fully grown at its birth late last year, of *Comprehensive Polymer Science (CPS)*. A coup, this: articles sometimes contain references to papers published in 1988, and are in other ways impressively up to date.

Polymers have evoked a vast human effort in invention, application and understanding, earning for researchers four Nobel prizes — a remarkably small number, perhaps because of the extent of interdependence on which advances have been founded. *Comprehensive Polymer Science* is introduced in Vol. 1 by an

authoritative historical survey by Allen (not many people know that the Worshipful Company of Horners of the City of London, which in the eleventh century dealt in thermally shaped horn for utensils, windows and lamps, is nowadays the Guild for industrialists in the plastics business). Faraday — who else — correctly identified C_3H_7 as the formula for the isoprene rubber unit, which was elaborated by Mark in the 1920s to the polymeric structure. Staudinger, also at that time, really established the polymer concept for both natural and synthetic materials, and resolution of the radical and condensation mechanisms followed, associated with the names of Flory, Carothers and their contemporaries. Among the great advances of the 1950s there stands out the discovery by Ziegler and Natta, of catalytic control of stereoregularity, yielding crystalline or ordered polymeric material. In overviews, the *Encyclopedia* contains, in addition to lists in Vols 1 and 10 respectively of the 593 publications by Mark, and the 364 by Flory, a highly scholarly entry on the history of polymer science in Vol. 7.

In the quest for treatments to compare and contrast, I first chose those on nuclear magnetic resonance. The close resemblance between the diagrams and systems is explained by F.A. Bovey's being the author of both (together with L.W. Jelinski in the *Encyclopedia*; Bovey was in fact also author of this chapter in the first edition). This sole overlap of authorship in the two series demonstrates the more austere scientific tautness in *CPS* against the more discursive expositions to be found in the *Encyclopedia*. Both series have excellent accounts of polymerization mechanisms, and the characterization and properties of polymers, organic, inorganic and organometallic. In *CPS* silicones are in, silicates out — you have to draw a line somewhere. The *Encyclopedia* is not comparable here, having at the time of writing only got up to "Safety".

As further examples, the articles on neutron scattering by G.D. Wignall in the *Encyclopedia* and by D.M. Sadler in *CPS* are equally informative and useful, the former providing introductory complementarity while managing within its greater length to deal with the main applications. The coverage of dielectric studies in *CPS* in all ways surpasses the brief *Encyclopedia* entry, but then I found the treatment of electroactive polymers more impressive in the *Encyclopedia*: Wegner, who is on the *CPS* editorial board, himself could have provided a magisterial contribution, but the corresponding chapter is in fact by Bloor (a physicist, notwithstanding the chemistry address erroneously assigned him). Wiley have done well in this regard to have produced *Electrical and Electronic Properties of Polymers*, a separate book



edited by Kroschwitz, which is a collection of the relevant chapters from the *Encyclopedia*. The collection is a useful adjunct to the literature in this new area, supplementing Skotheim's *Handbook of Conducting Polymers* and other monographs. The *CPS* article on electronics applications includes the fashionable phrase 'molecular electronics', with, note, a preponderance of Japanese references; that on electrochemical initiation I found disappointing and rather better done in the *Encyclopedia*.

The structure of *CPS* is better than that of the *Encyclopedia*, as it was bound to be, being of logical evolution and clearly draconian execution: several contributions are represented briefly in their logical places by a chapter heading and a note that the author's delay has meant relegation of his chapter to the back end of the book. Volume 1 starts with characterization methods, which are treated in as evolutionary an exposition as multi-authorship will allow. Volume 2 (*Properties*) deals among other things with chain statistics and conformations; with mechanical, hydrodynamic, electrical, optical and surface properties; and with polymer blends, fibres, crystals and glasses. Most of these topics can be found in the *Encyclopedia*, and their interspersal with more down to earth, often more technological entries, can be viewed according to taste as irksome and disruptive, or as relief from the sometimes severely physical expositions in *CPS*.

My own prejudice is that *CPS* is the more up to date in syntheses and mechanisms in Vols 3 and 4 — both on chain polymerization — and in Vol. 5, step polymerization. This impression, however, may simply arise from the sequential elaboration achieved in *CPS* which is lost in the alphabetical formula of the *Encyclopedia*. The enormous 1980s literature on ring-opening polymerization covered by *CPS* receives only a 24-page

article in the *Encyclopedia*.

I have done insufficient justice to the synthetic aspects of polymerization. But it is perhaps justifiable to generalize from the impressions noted thus far, that, in their general differences, each series diligently strives to fulfil its chosen designation. The *Encyclopedia* is the more descriptive, and provides the more accessible introduction (and with 'Engineering' in its title it does embrace a greater number of technical topics). *CPS* is more scientific, but as its subject matter is the very stuff of much of industry, it yet encompasses much of the technology; the final volume is entitled *Specialty Polymers and Polymer Processing*.

The proof-reading in *CPS* has slipped just a bit amongst the references (not the text), as might be excused in such a rush job. The *Encyclopedia* also is not faultless — under "Crystalline Polymers" it states "See Polymers, Crystalline", but you look in vain. The larger pages of *CPS*, together with its more crowded typesetting, and other lay-out differences, probably result in a 30–50 per cent greater word content per page than in the *Encyclopedia*; this is not an unqualified advantage. Each *Encyclopedia* volume begins with 18 pages on symbols and units, and a brief list of all the entries it includes; synonym titles are appropriately cross-referenced to the substantive articles. Each volume of *CPS* contains a list of the topics covered in all of the volumes, as well as its own good index. On top of that, there is a full series index in the final volume.

Workers in the field will need one of these series at hand, and will want the other available nearby. How widely embracing the *Encyclopedia*, how timely *CPS*! It's amazing the number of things one *does* need to know about polymers — if not penguins. □

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Inside the black box

Werner Israel

Kinetic Theory in the Expanding Universe. By Jeremy Bernstein. Cambridge University Press: 1988. Pp. 149. £22.50, \$37.50.

THROUGH much of its early history, the expanding Universe passed uneventfully through a succession of thermal equilibrium states, its equilibrium maintained by interactions which kept all constituents strongly coupled. But there were interludes when things became livelier. Many distinctive features observable today stem from episodes when equilibrium was upset because a declining reaction rate began to lag behind the expansion or the temperature dropped below the threshold for pair creation of some key particle. Most familiar is the three-minute episode of helium production at the end of the lepton era, but this kind of picture is pervasive in present-day cosmological theories.

Because a nonequilibrium episode is generally not brief compared to the expansion time, determination of the resulting cosmic abundances confronts the physicist with a genuine nonequilibrium problem, involving integration of an intricate system of reaction-rate equations. Understandably, the details have increasingly taken on a 'black-box' aspect, accessible only to specialists with elaborate computer codes. Bernstein's little book is a fine pedagogical introduction to this class of problems which demonstrates that this need not be so. By judicious approximations, one can derive analytical formulae that reproduce the computer results to within a few per cent. More important, they offer a ready way to estimate the effects of changing the many more-or-less uncertain parameters that enter the calculation: cross-sections and half-lives, the photon-to-baryon ratio, the degree of isotropy, the cosmological constant and modifications of general relativity.

The first section (35 pages) aims to provide thumbnail introductions to Friedmann–Robertson–Walker (FRW) models and to relativistic kinetic theory. It begins inauspiciously with a newtonian derivation of the FRW dynamical laws that is seriously flawed. (The proper starting point is not Newton's law of motion but the newtonian law of energy conservation: they are equivalent only for pressureless models.) The treatment of kinetic theory betrays no hint that general relativity is a geometrical theory, and the algebra is heavy-handed and opaque. A beginner, wishing to understand intuitively the "important remark" (p. 6) that an expanding Universe has no timelike

Killing vector, is none the wiser for being told that a Killing vector is a solution of a partial differential equation of unspecified provenance.

One should not make too much of such cavils. The book is otherwise a unique and valuable addition to the literature on cosmo-particle physics, and this introductory material is easily accessible elsewhere. Nevertheless, from an author with Jeremy Bernstein's gifts for lucid exposition one is entitled to expect more. Please Mr Bernstein: can we have a rewrite of these pages in the next edition? □

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Bedtime reading

Ian Oswald

Sleep and Dreaming. By Jacob Empson. Faber & Faber: 1989. Pp. 258. £12.99.

"THE favours of the strictest ladies will be wholly won for you, at the moment you become the sympathetic interpreter of their dreams" — this is just one wish-fulfilling idea from the many historical nuggets that Jacob Empson offers us in *Sleep and Dreaming*. Dreams have traditionally been regarded as prophetic, and from Empson we learn that 50 per cent of those possessed of the benefits of modern higher education still accept that dreams foretell the future.

Divergent-minded writers always have been able to let themselves go on dreams, but solid scientific knowledge about the physiology of sleep is recent. As in past centuries, however, the theorists of today reflect the ideas of their time. Empson gives a good summary of recent dream theories, such as those of Crick and Mitchison, or Hobson and McCarley, which ride upon the back of physiology. To my mind, Empson could have brought home more sharply to the reader that the new theories (alas, no more open to objective verification than those of past centuries) fail to take account of the continuum in mental life as it is experienced in day-dreaming, in drowsiness and in all the stages of sleep. The theorists over-emphasize visual imagery, rather than recognize the dream as an experience of living in a fantasy world, and they forget that people blind from birth dream as much as anyone else.

Dreams are not eye movements, nor electrical discharges from the hind brain. True enough, if people are wakened from periods of rapid eye movement (REM or paradoxical) sleep, there is a good chance that a dream will be described, but it is

only twice the chance of an equally rich dream being described after rousing someone who had merely become drowsy. The most intense of all dreams are night terrors, and they are not related in time to REM sleep.

In order for us to lay down information in our memory store we have to pay attention. While we sleep we are not keenly paying attention, and afterwards we remember hardly anything of the dreams that occupied a large part of the night. As Empson says, if dreaming really were a means of finding solutions to life's problems, as some would claim, it would be a bit pointless when the dreams are almost entirely forgotten within minutes.

Readers will not find the book spoiled by attempts at over-inclusiveness, nor will they be wearied by any personal hobby horse. There is sensible advice to those who are dissatisfied with their sleep, to relax and forgive their enemies, and to get up earlier in the morning in order to make sure of swift falling asleep at night. I was, however, foxed by the remark about infants who wake their parents at night: "It is important not to allow these sleep problems to continue after the age of two". I'm afraid that some child psychologists, who make claims of being able to help desperate parents, do not bring with them evidence from parallel-group controlled trials. Normal maturation means that most children become good sleepers by school age without 'treatment', but they will not oblige by promptly doing so at the third birthday. Empson's compact book is well written, the best of its kind, and one that deserves to be widely bought. □

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• A book that appeared after preparation of the review of Empson's monograph is *Sleep* by J. Allan Hobson, a beautifully produced volume in the Scientific American Library series (W.H. Freeman; \$32.95, £14.95). Hobson has been a distinguished researcher into the activity of cells in the brainstem of laboratory animals. Here he writes in a highly personal way, the first page setting the tone: "the study of sleep and especially dreaming is changing humankind's view of itself". Moreover, "The new psychology of dreaming could help all of us, mentally ill or sane, function better". Assertions such as this are recurrent. The mechanism of sleep is that "the thalamus and cortex become susceptible to the normal loss of consciousness": in my view this statement would be no more comprehensible to most neurophysiologists than to psychologists. I don't think human sleep problems are really explained by "the ratio of aminergic to cholinergic neuronal firing level". The photographs of sleeping animals are lovely, but otherwise I was disappointed. Ian Oswald

Preservation of organic matter during salinity excursions

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Sediment in a core recovered from the western Mediterranean contains anomalously high concentrations of dissolved strontium, calcium, chloride and sulphate. These unusual results mirror the effects of early diagenesis in evaporitic basins, and a new model is proposed to explain anomalies in the accumulation of organic carbon and its isotopic composition. Such anomalies may result from increased but preferential preservation of organics brought about by changes in the microbial community during salinity excursions. It follows that the down-core record of sapropel formation in the Mediterranean contains a history of bottom-water salinity excursions.

ORGANIC carbon contents of buried marine sediments are the net result of detrital input from land and production in surface waters, modified by oxidation in the water column and diagenesis in sediments. To a large extent, fluctuations in the amount of organic carbon arriving at the sediment interface are buffered by microbially mediated reactions in the first few centimetres of sediment¹. The amount of carbon preserved in sediments is therefore rather insensitive to changes in sedimentation rate², in fact so much so that the organic carbon content of deep-sea sediments is typically invariant with depth and less than 1% of the total.

Sapropels are exceptions to this as they occur in pelagic sediments and contain more than 2% organic carbon. Carbon isotope studies³ have shown that the organic matter in sapropels is mainly of marine origin, which suggests that most sapropels are not produced by large increases in the supply of terrestrial carbon. Two diverse theories persist, both claiming to explain sapropelic, organic-rich facies: (1) increased production in surface waters⁴ and (2) increased preservation resulting from bottom-water anoxia⁵. The case for productivity has been bolstered by the discovery of a remarkably strong correlation between positive anomalies in the ratio of barium to aluminium in these layers and anomalies in $\delta^{13}\text{C}$ (ref. 6). This argument relies on the premise that barium accumulation is a measure of surface-water productivity^{7,8}. Evidence for control by anoxia includes

observations that organic-rich layers are usually laminated and devoid of benthic fauna⁹, indicating an absence of bioturbation^{10,11}.

The process we propose here for the formation of sapropelic layers requires neither anoxia nor increased primary production. It is simply a disruption of normal sediment diagenesis by alteration of the benthic community as a direct result of excursions in bottom-water salinity. These excursions may result from *in situ* dissolution of an evaporite deposit or advection of bottom waters of greater salinity.

Pore-water chemistry

Table 1 lists results of pore-water analyses from core N07A collected on the Rhone fan in the extreme western Mediterranean just north-east of Banyuls, France, in 360 m of water. The fan is characterized by a series of steep canyons that cut into the shelf. Pore waters were analysed for eleven cores raised from seven sites scattered across the shelf, through the canyons and out to abyssal depths.

Sediment from these cores was separated, centrifuged and filtered onboard the *G. Petit* (Laboratoire Arago de Banyuls) to obtain samples of interstitial water. Sulphate and calcium were measured by ion chromatography and flame atomic-absorption spectrophotometry, respectively. Chloride was determined by potentiometric titration. Barium and strontium concentrations were measured with the VG PlasmaQuad 1 inductively coupled plasma mass spectrometer (ICPMS) at MIT. Barium was determined with the PlasmaQuad using an isotope-dilution technique¹², whereas strontium was determined on the same instrument by internal standardization to bottom water at this site (MED 300). For comparison, strontium was also measured by a standard column-isotope dilution procedure (IDMS) on a thermal-ionization mass spectrometer. These column fractions were also used to measure the isotopic compositions. The ratios of $^{87}\text{Sr}/^{86}\text{Sr}$ were normalized to a $^{86}\text{Sr}/^{88}\text{Sr}$ ratio of 0.1194 and an E & A standard value of 0.7080.

Of the eleven cores analysed, N07A was the only core from canyon station 7 and the only one to exhibit the types of anomalies discussed here. Strontium concentrations in this core increase linearly with depth to levels that are 2.5 times bottom water in just 16 cm (Fig. 1a). This result indicates some unusual process deeper in the sediments at this site, as levels of pore-water strontium in the other cores collected from this area were the same as the levels in bottom water, which is consistent with

TABLE 1 Pore-water chemistry, core N07A

Sample	Barium (nmol kg ⁻¹)	Calcium (mmol kg ⁻¹)	Sulphate (mmol kg ⁻¹)	Chloride (mmol kg ⁻¹)	Strontium ($\mu\text{mol kg}^{-1}$)		$(^{87}\text{Sr}/^{86}\text{Sr})_{\text{N}}$	$\pm 2\sigma$
					IDMS	ICPMS		
MED 300	80	11.3	31.0	600	100.4	(100.4)	0.709174	67
0-3 cm	207	13.9	31.7	634	112.8	108	0.709105	39
3-6	263	21.6	35.8	767	161.0	158	0.709086	33
6-8	256	23.5	36.9	819	175.0	169	0.709062	43
8-10	266	29.1	38.8	835	207	206	0.709043	33
13-16	304	37.0	39.9	1,150	248	246	0.709005	38

* Normalized $^{87}\text{Sr}/^{86}\text{Sr}$ ratios (denoted by N).

pore-water data from other parts of the Mediterranean¹³.

Several processes have been identified as possible causes of substantial increases in pore-water strontium: (1) alteration of volcanic material; (2) carbonate recrystallization; (3) dissolution of evaporites. There is substantial evidence that this western Mediterranean site is being affected by an evaporite. This evidence consists of predictable increases in concentrations of dissolved strontium (Fig. 1a) and dissolved calcium (Fig. 2b), with the $\Delta\text{Sr}/\Delta\text{Ca}$ ratio (Fig. 2c) expected by comparison with Deep Sea Drilling Project (DSDP) results¹⁵, and decreases in $\delta^{87}\text{Sr}$ (Fig. 1b) and commensurate increases in chloride (Fig. 2a) and sulphate (Fig. 3b). These observations are strongly suggestive of dissolution from an evaporitic layer of gypsum or anhydrite, probably of Messinian age (~ 5.2 Myr BP).

Pore-water gradients near evaporitic facies are complicated¹⁵. Calcium and strontium increase linearly with depth down this short core, but they may be extremely nonlinear at greater depths. On the other hand, chloride should be less reactive and it also increased linearly down this core (Fig. 2a). If the chloride gradient is extrapolated to 5,000 mM, the level observed for pore fluids just above an evaporitic layer¹⁵, the evaporite at station 7 occurs at 120 cm.

The strontium isotope data are consistent with this depth, in that a plot of $\delta^{87}\text{Sr}$ against $1/\text{Sr}$ for these pore waters shows the expected curvature (Fig. 1c). Moreover, if 'excess' strontium (Fig. 2b) of variable isotopic composition is mixed with seawater strontium to produce this curve, the 'best-fit' evaporitic endmember composition is identical to Messinian sea water ($\delta^{87}\text{Sr} =$

0.70891–0.70902) but somewhat more radiogenic than Ocean Drilling Program (ODP) evaporites from the Tyrrhenian Sea¹⁶ ($\delta^{87}\text{Sr} = 0.70861\text{--}0.70886$).

The pore-water data given here are the first evidence for evaporites in this area. Salt diapirs are common in the western Mediterranean but at greater water and sub-floor depths¹⁷. The site of core N07A is apparently above an evaporitic outcrop associated with this canyon. Outcropping here is not surprising given the extent of sediment scouring by turbidity currents expected at such a location.

Effects of early diagenesis

Early diagenesis in evaporitic basins affects the system mainly in three ways: (1) by altering the chemical composition of the evaporite; (2) by 'ageing' the strontium isotopic composition of interstitial and overlying waters; (3) by perturbing normal organic diagenesis. This last point will be discussed in the following section.

Upper Miocene gypsums and anhydrites recovered during ODP Leg 107, holes 652A, 653B and 654A in the Tyrrhenian Sea¹⁶, contain less strontium than freshly precipitated crystals of these minerals^{18,19}. The data presented here would suggest that diagenesis could account for this discrepancy. Certainly, the strontium fluxes predicted from pore-water gradients at this site (Fig. 1) are large enough to produce substantial changes in an evaporitic facies. Multiplying the concentration gradient of dissolved strontium by a diffusion coefficient of $1.5 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ results in a calculated diffusive flux of $0.5 \mu\text{mol Sr cm}^{-2} \text{ yr}^{-1}$ upwards through the sediment column. The strontium content of fresh gypsum or anhydrite is $\sim 2,000 \text{ p.p.m.}$ ^{18,19}, or twice that found in Tyrrhenian evaporites¹⁶. At the rate exhibited in core N07A, it would take 53 years to diagenetically alter 1 cm of evaporite to this reduced Sr content, that is, 3 m of evaporite would be affected in the time it took to accumulate 15 cm of sediment (at a sedimentation rate of 1 cm yr^{-1}). This calculation ignores porosity effects. Nevertheless, it does demonstrate that evaporites can be substantially altered by early diagenesis soon after being deposited or after being exposed by loss of sediment.

This diagenetic alteration is not congruent dissolution. The Sr/Ca mole ratio in these pore waters is more than double the ratio in fresh anhydrites or gypsums. It seems reasonable to assume that fractionation of strontium from calcium would occur during this process if much of the strontium in gypsum or anhydrite exists as celestite (SrSO_4)²⁰. Hence, it seems likely that incongruent dissolution during early diagenesis was responsible for the extremely low Sr/Ca ratios observed in Tyrrhenian evaporites, which are three to nine times lower than the pore-water ratio. Preferential dissolution of Messinian evaporites during sediment diagenesis was also invoked by ten Haven *et al.*¹³ to explain interstitial profiles from the eastern Mediterranean.

Of course, recycling of evaporitic strontium through interstitial waters affects the isotopic signatures of pore waters as well as that of overlying bottom waters. The right-hand scale in Fig. 1b denotes strontium ages assigned to pore waters at these depth intervals from the seawater strontium-age curve published by Elderfield²¹. This geochronology demonstrates that carbonates equilibrating with dissolved strontium at this site will possess an apparent strontium age much older than contemporaneous sea water. For example, benthic foraminifera living several centimetres below the interface²² at this site today would acquire a strontium age of more than 1 Myr BP.

Preservation of organic matter

The pore-water chemistry of core N07A mimics early-stage diagenesis in an evaporitic basin, with one major difference. The salinity of pore waters at the sea/sediment interface would have been much higher during evaporitic times, resulting directly from evaporation or indirectly from dissolution of evaporites.

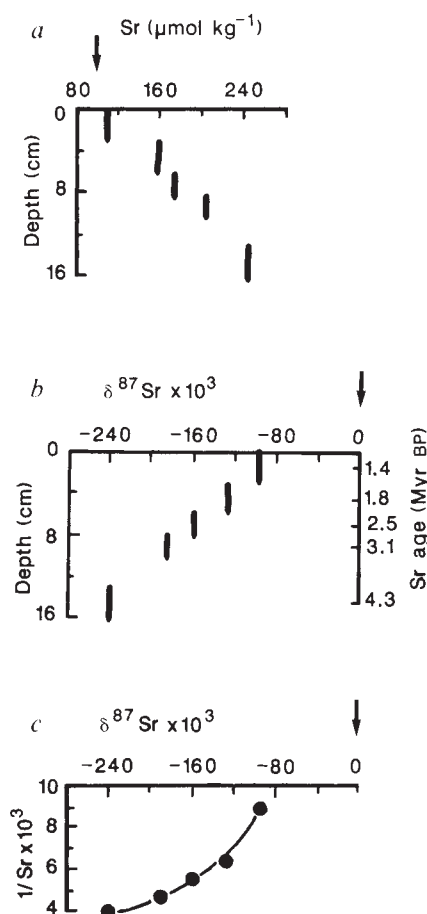


FIG. 1. Profiles of interstitial strontium (a) and its isotopic composition (b). The arrows indicate bottom-water (300 m) values. $\delta^{87}\text{Sr}$ is defined as the normalized 87/86 ratio for pore water divided by the normalized 87/86 ratio for bottom water minus one, multiplied by 1,000. c, Isotopic compositions against $1/\text{Sr}$.

For example, the salinity at the interface of core N07A today is 5.7% higher than present-day bottom water. If the top 10 cm of sediment were removed, the salinity at the interface would be 74‰, almost double that of bottom water.

The situation during evaporitic times would have been similar to present-day Bannock or Tyro Basins in the eastern Mediterranean, where the bottom is covered by a hypersaline brine produced by the dissolution of Messinian evaporites^{13,23,24}. These dense brines inhibit exchange with overlying waters so that anoxic pools are established within these basins.

Sediments in these hypersaline areas accumulate organic carbon at an anomalously fast rate²⁵. Such accumulation in these settings may not result from anoxia or changes in surface-water productivity but from shifts in the nature of early diagenesis. This argument rests on two premises: (1) increases in productivity are not required as organic carbon is being supplied to sediments fast enough under normal circumstances to support the observed anomalies in organic-carbon accumulation; (2) the quantity and quality of microbial activity normally responsible for consuming organics at the sediment interface is severely disrupted by excursions in salinity.

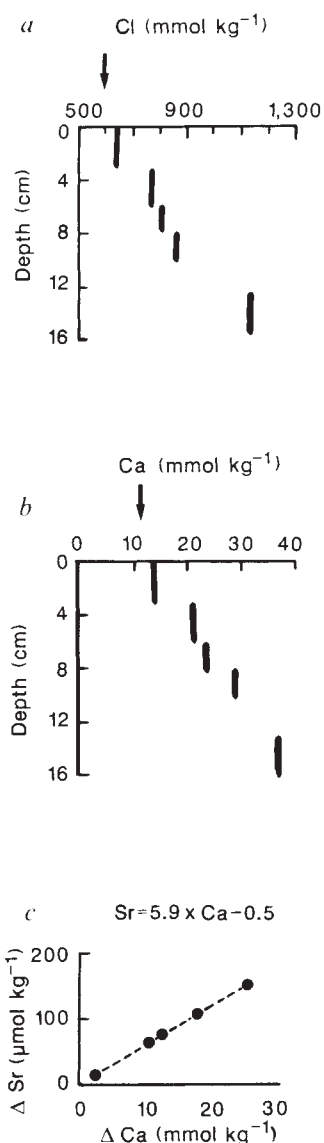


FIG. 2 a, Dissolved chloride concentrations in core N07A plotted against depth. b, Dissolved calcium against depth. c, Pore-water strontium minus bottom-water strontium plotted against equivalent data for calcium.

Measurements of rainout rates through the water column and accumulation at the sea/sediment interface are both consistent with the view that carbon is being supplied to sediments fast enough to support organic-rich layers. Vertical fluxes of carbon through sea water vary widely but are usually between 1 and 100 g C m⁻² yr⁻¹ (ref. 2). Sapropels and sediments in present-day hypersaline basins accumulate carbon within this range (see, for example, ref. 25). Oligotrophic sites such as the Sargasso Sea²⁶ give values at the lower end of this range; productive zones such as the Peru upwelling area² are on the high side. Carbon fluxes measured at other places fall between these extremes. For example, a flux of 30 g C m⁻² yr⁻¹ has been reported²⁷ for a site in the eastern Mediterranean. In addition to the vertical flux of carbon from the surface, basin sediments often receive an additional flux of carbon from lateral transport²⁸. Thus it appears that the supply of organic carbon from vertical and lateral transport could account for carbon accumulations in organic-rich layers, but only if most carbon was preserved after reaching the sediment interface.

This conclusion is supported further by examining what is observed in a normal marine sequence. Several authors²⁸⁻³¹ have reported a maximum in particulate organic carbon (POC) near the interface of carefully collected and carefully separated cores. This POC anomaly is restricted to the top few centimetres of sediment, which can be enriched in carbon by as much as 2-3%. The extent of this carbon enrichment seems to be limited only by the sampling interval, even when sediments are sectioned on a millimetre scale³¹. Indexes of microbial activity such as ATP (adenosine triphosphate) biomass coincide with this surficial enrichment²⁸. Carbon deposited at the interface is mixed down from the 'fluff' layer by macrofauna, accounting for the width of the anomaly in POC.

In the absence of macrofauna, bioturbation would cease. The POC, which is spread out over the top 1-2 cm under normal circumstances, would accumulate instead right at the interface, resulting in interfacial sediments containing 5% or more organic carbon. This change alone would result in the deposition of laminated layers like sapropels; indeed, lamination has been used as evidence for anoxia^{9,10}. However, although it prevents labile organics from being mixed into the sediment, anoxia does not diminish net microbial metabolism and may even increase this activity³². Thus, most organic carbon in this case will be consumed by anaerobic bacteria. Indeed, most labile organic matter in near-shore sediments is consumed under suboxic or anoxic conditions (see, for example, ref. 33). The net result of simply eliminating macrofauna by inducing anoxia in bottom waters will be laminations but little change in the accumulation rate of organic carbon. It seems more likely that the increase in preservation of organic matter necessary to produce a sapropel-like layer is brought about not simply by stagnation of the overlying water column but by coincident changes in the entire benthic population.

Marine microbes require cations to maintain proper osmotic balance. Not surprisingly, aerobic and anaerobic strains of halophilic marine bacteria attain their maximum numbers and greatest metabolic efficiencies at seawater salinities (see, for example, ref. 34). What is most interesting is that many microbial growth curves break sharply at higher salinities, so that the abundances of these strains are reduced by as much as 40% when salinity is increased from 35‰ to 40‰. Higher salinities can have even more dramatic effects. Recent measurements of growth and respiration in the Orca Basin, Gulf of Mexico³⁵, indicate that the microbial community in the brines there are completely inactive. Interestingly, bottom-water salinities in the Mediterranean seem to be poised for a microbial salinity crisis. This observation may explain why sapropels are common here and why they occur more frequently in the saltier eastern sea.

Communal and metabolic changes in the microbial population brought on by excursions in salinity would also account for isotopic shifts in the organic carbon found in organic-rich

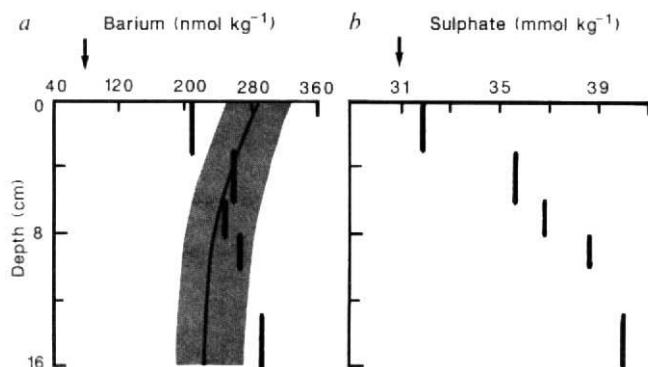


FIG. 3 Interstitial barium (a) and sulphate (b) for core N07A. The curved line is saturation with barite and the shaded area indicates the uncertainty inherent in these calculations (ref. 41).

layers⁶. Osmotic stress resulting from increased salt induces cellular adaptations (see, for example, refs 36 and 37) which result in a microbial population which functions with a lower metabolic efficiency (H. Jannasch, personal communication). The net result of this change will be a stressed microbial community with a preference for a different organic fraction than the normal population. Selective preservation would then produce POC with an isotopic signature shifted several per mil from the carbon that accumulates normally³⁸. A change of this magnitude is the same as the isotope shift recorded in sapropels.

A salt effect would also explain the impressive correlation of carbon isotope anomalies with anomalous Ba/Al ratios in organic-rich layers. Barium in marine pore waters exceeds saturation with barite within a few centimetres of the interface under normal circumstances (G.P.K., unpublished data). Figure 3a illustrates that the pore waters in core N07A are no exception. These pore waters become saturated with barite a few centimetres below the interface. If additional sulphate were added to this sediment column from below or above, this barite horizon would migrate towards the sea/sediment interface or even into bottom waters. In either case, barite would accumulate at the organic-rich interface, thus accounting for the good correlation between sedimentary barium and carbon. This view is supported by barium and sulphate data for the Tyro and Bannock basins.

Bottom water from the Tyro Basin contains less sulphate (51.4 mM) and more barium (490 nM) than bottom water from the Bannock Basin (138 mM sulphate, 130 nM barium) (G.P.K., unpublished data). Increased sulphate levels during sapropel formation would not only account for the 'salting out' of barium but would also explain why gypsum is often a component of sapropelic layers³⁹.

If carbon and barium respond to changes in salinity, then the down-core record of sapropel formation in the Mediterranean contains a history of excursions in bottom-water salinities. It follows from this argument that more subtle but coincident changes in barium and carbon at other sites (see, for example, ref. 40) may be indicative of shifts not in palaeoproductivity or palaeoredox but in palaeosalinity.

Curtailed microbial activity as a direct result of increases in salinity would seem to explain several observations commonly associated with sediments in evaporitic basins. These include: large excursions in organic content, absence of benthic fauna in sapropelic layers, laminations, shifts in the isotopic composition of carbon, common occurrence of gypsum in sapropels, frequent discoloration of evaporitic facies by organics, occasional occurrence of oil deposits above salt diapirs, high barium contents of organic-rich layers and rapid accumulation of organic carbon in hypersaline basins. □

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Cyclin synthesis drives the early embryonic cell cycle

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We have produced extracts of frog eggs that can perform multiple cell cycles *in vitro*. Destruction of the endogenous messenger RNA arrests the extracts in interphase. The addition of exogenous cyclin mRNA is sufficient to produce multiple cell cycles. The newly synthesized cyclin protein accumulates during each interphase and is degraded at the end of each mitosis.

THE early embryonic cell cycle of many organisms is a rapid alternation of interphase and mitotic states. The transition from interphase to mitosis is induced by the appearance of an activity named maturation promoting factor (MPF)¹⁻³, which is highly conserved⁴. The cytoplasm of meiotic and mitotic cells of a wide range of eukaryotes contains MPF activity, which can be measured by its ability to induce maturation in *Xenopus* oocytes. MPF is now believed to be a protein kinase that initiates a cascade of reactions which lead ultimately to nuclear envelope breakdown, chromosome condensation and the assembly of the mitotic spindle⁵. This view is supported by the recent finding that one of the subunits of MPF is homologous to the gene product of the *cdc2* gene of *Schizosaccharomyces pombe*, whose activity is required for entry into mitosis⁶⁻⁸. The gene product of the *cdc2* gene is a protein of relative molecular mass 34,000, referred to as p34^{cdc2}, which shares homology with known protein kinases. Immunoprecipitates made from yeast, *Xenopus* and starfish using anti-p34^{cdc2} antisera have protein kinase activity on a number of substrates including histone H1 (refs 7-11).

The fluctuation of MPF activity in *Xenopus* oocytes, eggs and embryos is shown schematically in Fig. 1. Fully grown immature oocytes are arrested in prophase of meiosis I with no detectable MPF activity. Secretion of progesterone by the follicle cells that surround the oocyte induces the post-translational activation of an inactive form of MPF and meiosis I (ref. 3). MPF activity then falls before rising again at the onset of meiosis II. The mature oocytes (which pass down the oviduct and emerge as unfertilized eggs) arrest at metaphase of meiosis II by virtue of a calcium-sensitive activity named cytosolic factor (CSF), which stabilizes MPF activity¹². Fertilization or artificial activation triggers a rise in the intracellular calcium level, the inactivation of CSF, a decline in MPF activity and entry into interphase of the first mitotic cell cycle. At the end of each interphase MPF activity rises transiently, leading to the induction of mitosis; as MPF activity falls the cell enters the next interphase³.

In a wide variety of organisms protein synthesis is required for the appearance of active MPF in meiosis II and in mitosis^{3,13-15}. In the mitotic cell cycles protein synthesis is required during each and every interphase for the induction of the subsequent mitosis. Because early embryos have large stores of the enzymes and structural proteins required for DNA synthesis and mitosis, the requirement for protein synthesis is likely to represent the synthesis of important cell-cycle regulatory molecules. One attractive candidate for a newly synthesized inducer of mitosis is cyclin^{15,16}. The cyclins were identified as embryonic proteins that accumulate during interphase and decline precipitously in abundance during mitosis¹⁵. Cyclin abundance is regulated by controlling the half life of the protein;

it is long during interphase and declines during mitosis. A role for cyclin in regulating mitosis was suggested by experiments which show that cyclin mRNA induces maturation in *Xenopus* oocytes^{16,17}. Cyclins have now been identified in many organisms, including sea urchins¹⁷, clams¹⁶, starfish¹⁸, *Xenopus*¹⁹, *Drosophila*²⁰ and *S. pombe*²¹⁻²⁴, and have been divided into two classes, A and B, on the basis of their sizes¹⁵, kinetics of appearance¹⁵ and sequence homology¹⁹.

To study the role of cyclin in the mitotic cell cycle, we turned to extracts of amphibian eggs that would perform the key reactions of the cell cycle²⁵⁻³¹. We have developed procedures for producing *Xenopus* extracts that undergo multiple cell cycles, extending the work of Lohka and Masui, who produced a single cell cycle *in vitro*²⁵. We have used these extracts to show that cyclin is the only newly synthesized protein required to induce mitosis.

Xenopus cyclin *in vivo*

We looked for *Xenopus* cyclins in eggs that had been released from the CSF-mediated metaphase arrest by electrical activation. Shortly after activation the eggs were injected with [³⁵S]methionine, incubated for various times before lysis, and the newly synthesized proteins analysed on SDS-polyacrylamide gels. A closely spaced set of bands, running with apparent relative molecular mass (*M_r*) of 55,000 (55K), increased in intensity throughout the first cell cycle but then decreased dramatically between 60 and 75 min after activation. This corresponds to the time during the first cell cycle when MPF activity would normally fall at the onset of anaphase. These bands then increased in intensity, only to decline again at the time (85-95 min) when MPF would normally fall (data not shown). The pattern of accumulation and disappearance of the 55K bands suggests that these proteins are cyclins, although they are much less abundant than their counterparts in clams and sea urchins.

In vitro cell cycle

We prepared *Xenopus* egg extracts that undergo multiple cell cycles *in vitro*. Concentrated extracts were prepared from electrically activated eggs that had been washed in a buffer designed to mimic the ionic composition of egg cytoplasm, crushed and fractionated by centrifugation. Demembrated sperm nuclei and an ATP regenerating system were then added to the concen-

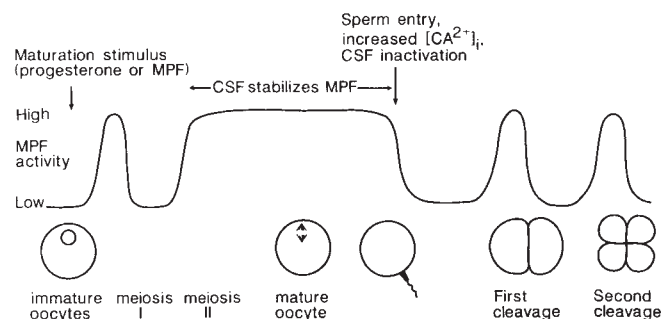


FIG. 1 MPF levels during early *Xenopus* embryonic development. The fluctuation in MPF levels as an immature oocyte passes through meiotic maturation, fertilization and the first two mitotic cell cycles is shown. For further details see the text.

trated cytoplasmic extract. The extracts we have produced have cell-cycle times varying between 35 and 55 min, compared with *in vivo* cycle times of 25–30 min. These extracts, which we refer to as cycling extracts, routinely produce at least three complete cell cycles.

We monitored the progress of the cell cycle in extracts by examining the morphology of the added sperm nuclei in fixed

samples stained with a DNA-binding fluorescent dye. We could distinguish five states of the nuclei: interphase, prophase, mitosis and early and late telophase. In the experiment shown in Fig. 2a, the sperm nuclei decondensed to form interphase nuclei after 30 min incubation. The nuclear envelope was visible both by phase contrast and immunofluorescence with anti-lamin antibodies (data not shown). At 45 min the nuclei were in prophase,

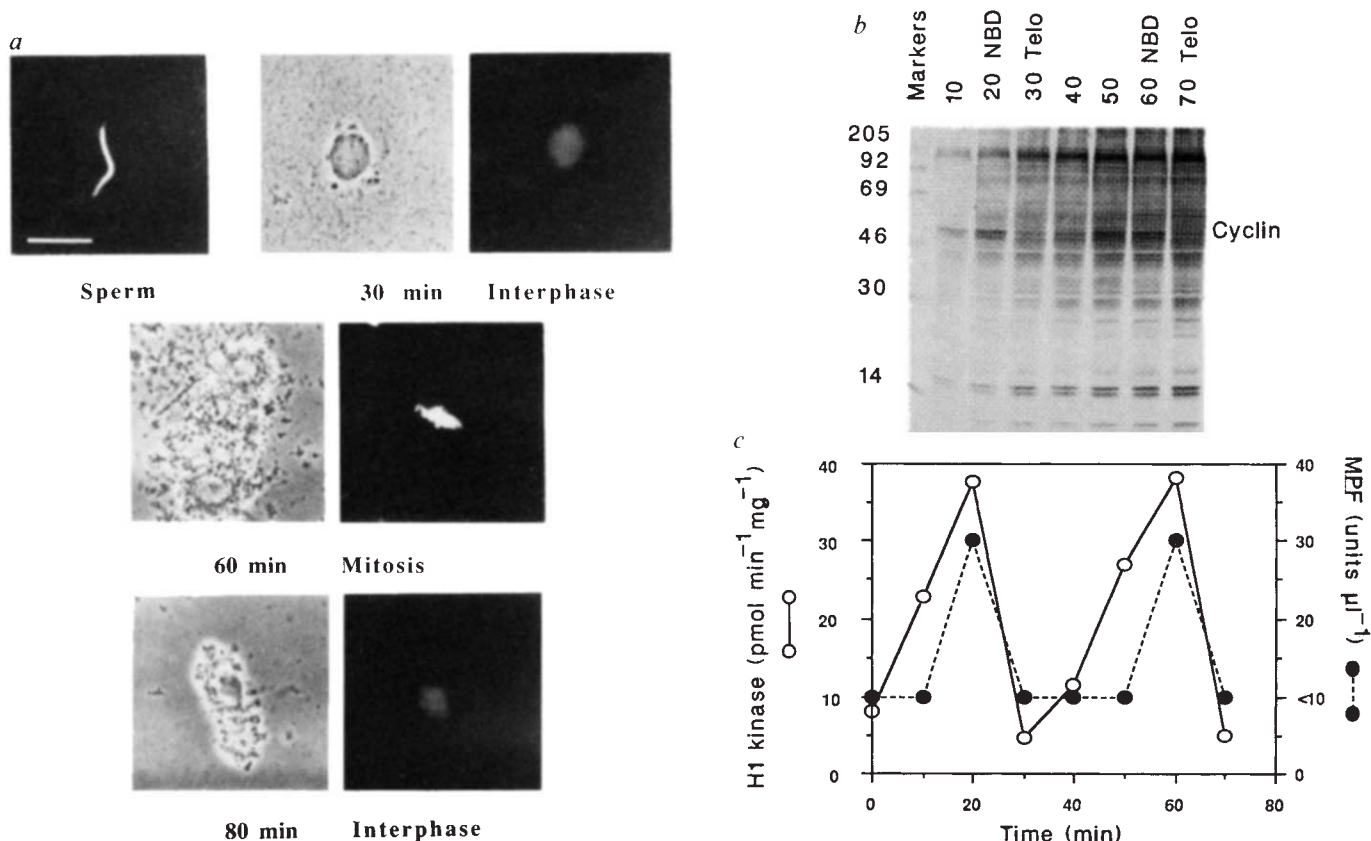


Fig. 2 Behaviour of *in vitro* cell-cycle extracts. *a*, Nuclear morphology at the indicated times of an *in vitro* cell-cycle extract to which sperm nuclei had been added at time zero. Each pair of images consists of a phase contrast image on the left and a fluorescent image of the DNA-binding dye, Hoechst 33342, on the right. Only the fluorescent image of the intact sperm is shown. Scale bar, 20 μm. *b*, Autoradiograph showing the labelled proteins synthesized in an *in vitro* cell-cycle extract to which [³⁵S]methionine had been added at time zero. Samples were taken at the indicated times and either diluted in sample buffer and run on a 12.5% polyacrylamide gel, or fixed for determination of the nuclear morphology. The times of nuclear envelope breakdown (NBD) and telophase (Telo), the positions of the molecular weight markers (in thousands) and the set of cyclin bands are indicated. *c*, The levels of H1 kinase (○—○) and MPF (●—●) activity during incubation of the extract shown in *b*.

METHODS. Cycling extracts were prepared as follows. Frogs were induced to ovulate and eggs were collected by squeezing into MMR (100 mM NaCl, 2 mM KCl, 1 mM MgCl₂, 2 mM CaCl₂, 0.1 mM NaEGTA, 5 mM NaHEPES, pH 7.8)⁴⁶ and dejellied with 2% cysteine, pH 7.9, and then electrically activated in 0.2 × MMR by two 1-s pulses of 12 V a.c. The activated eggs were washed four times with XB (100 mM KCl, 1 mM MgCl₂, 0.1 mM CaCl₂, 10 mM KHEPES pH 7.7, 50 mM sucrose) and then twice with XB containing 10 μg ml⁻¹ each of leupeptin, chymostatin and pepstatin. Finally, the activated eggs were transferred with a minimal volume of XB to a 5 ml polycarbonate centrifuge tube containing 1 ml of XB plus protease inhibitors containing 100 μg ml⁻¹ of cytochalasin B. Ten minutes after activation any residual buffer was removed and the eggs were overlaid with 1 ml Versilube F-50 oil (General Electric, ρ = 1.03 g ml⁻¹) and spun at 200g for 1 min. The buffer that had been displaced by the oil was then removed and at 15 min after activation the eggs were chilled in an ice bath before being spun for 10 min at 15,000g at 2 °C. The cytoplasmic layer was collected by side puncture and the following ingredients were added: MgATP to 1 mM, creatine phosphate to 10 mM, EGTA, pH 7.7, to 0.2 mM and leupeptin, chymostatin, pepstatin and cytochalasin B to 10 μg ml⁻¹ each. The extract was then spun again at

15,000g for 15 min at 2 °C to remove residual yolk and pigment granules. This extract was stored on ice and used within three hours. All the experiments in this paper were performed with fresh extracts and the final volume of added components never exceeded 20% of the volume of cytoplasmic extract. Sperm nuclei were prepared by a slight modification of the method described by Gurdon⁴⁷, stored in 30% glycerol at -70 °C and used at a final concentration of 10⁵ per ml in the extract. All incubations were performed at 23 °C. Samples were analysed for morphology by spotting 1 μl extract on a microscope slide and then adding 4 μl fixative (MMR containing 50% glycerol (w/v), 10% formalin and 1 μg ml⁻¹ Hoechst 33342) before squashing the drop with a coverslip and examining the nuclei by phase contrast and fluorescent microscopy. MPF was assayed by diluting samples twofold into EB (80 mM potassium β-glycerophosphate, 15 mM MgCl₂, 20 mM potassium EGTA, adjusted to pH 7.3 after mixing the components) and assaying them for MPF activity in cycloheximide-treated immature oocytes as described³. One MPF unit is the amount of MPF activity which when injected in a volume of 50 nl will induce germinal vesicle breakdown in 50% of injected oocytes. H1 kinase activity was assayed by diluting samples fiftyfold into EB and assaying for H1 kinase activity by adding 10 μl to 6 μl containing 1 mg ml⁻¹ of calf thymus histones (a gift of J. Minden⁴⁸), and 1 mM ATP, 0.25 μCi μl⁻¹ [³²P]ATP. The phosphorylated H1 kinase was run on 5–15% gradient polyacrylamide gels and the amount of incorporated phosphate quantified by cutting out and counting the gel bands or by densitometry; H1 kinase activity is expressed as pmol incorporated phosphate min⁻¹ mg⁻¹ of protein (for counted gel bands) or arbitrary units (for samples assayed by densitometry). All the MPF and H1 kinase assays in this paper were performed on samples that had been diluted into EB and then frozen in liquid nitrogen. To analyse the pattern of protein synthesis [³⁵S]methionine was added to a final concentration of 0.4 mCi ml⁻¹ and samples were taken, diluted tenfold into sample buffer and run on 12.5% polyacrylamide gels⁴⁹. Detailed protocols for the preparation of cell-cycle extracts are available on request.

which is characterized by the start of chromosome condensation and the further swelling of the nucleus. Nuclear breakdown occurred by 60 min and clusters of condensed chromosomes were seen. Nuclear breakdown and chromosome condensation occurred in all extracts, but only some extracts formed well defined mitotic spindles. At 70 min the chromosomes had started to decondense into the individual mini-nuclei or karyomeres characteristic of early telophase, and by 80 min interphase nuclei were present once more. At 100 min the nuclei were in prophase and a second round of nuclear breakdown followed at 110 min. The synchrony of the nuclei in these extracts was good: at most time points all the nuclei were in the same morphological stage and no time point contained nuclei in more than two stages. *In vitro*, as *in vivo*, protein synthesis was required in each interphase to allow the occurrence of the next round of nuclear breakdown (data not shown).

The pattern of protein synthesis in cycling extracts was monitored by adding [35 S]methionine at the start of the reaction and withdrawing samples at intervals during two cell cycles and analysing them on SDS gels. Figure 2*b* shows the behaviour of the cyclin bands *in vitro*, which is essentially identical to that seen *in vivo*. The intensity of the cyclin bands increased throughout the first interphase and then declined dramatically between nuclear breakdown and the onset of telophase. During the second interphase the intensity of these bands again increased, only to fall again after the second round of nuclear breakdown. These bands are indeed the translation products of the *Xenopus* cyclin B genes that have been cloned on the basis of their homology with sea urchin cyclin: treatment with RNase H and anti-sense oligonucleotides derived from the cloned cyclins destroyed the mRNAs that encode these bands (J. Minshull, T. Hunt, A. W. M. and M. W. K., unpublished data). The two primary cyclin translation products undergo post-translational modification to yield as many as five closely spaced bands on gels¹⁹.

To demonstrate that biochemical events occurring *in vivo* also occur in cell-cycle extracts, we monitored DNA replication and the levels of both MPF and H1 kinase activity, which recent evidence suggests is very closely related to MPF^{7,9,11}. Figure 2*c* shows that MPF activity appeared at the time of nuclear breakdown and was undetectable by early telophase. Like MPF, H1 kinase activity peaked at the time of nuclear breakdown and was dramatically diminished by telophase, although appreciable activity was detectable in late interphase and prophase, when MPF was undetectable. This apparent discrepancy may simply reflect the high threshold for the MPF assay. We have also shown that DNA replication only occurs during interphase (data not shown), as previously shown in other *Xenopus* extracts^{26,28}.

Cyclin induces mitosis

To test whether cyclin synthesis in the absence of other protein synthesis can induce mitosis, we added cyclin mRNA to cell-cycle extracts whose endogenous mRNA had been destroyed. We made such extracts, which we call mRNA-dependent extracts, by using pancreatic RNase to destroy the endogenous mRNA in extracts prepared from activated eggs and then inhibiting the RNase with placental RNase inhibitor, RNasin³². Figure 3*a* compares the pattern of protein synthesis in an mRNA-dependent extract, to which no exogenous mRNA had been added, with that in a mock treated extract to which the RNase inhibitor, but no RNase, had been added. In the mock treated extract cyclin accumulated until 30 min, when breakdown of the nuclear envelope occurred. By 40 min the cyclin bands had declined in intensity and telophase had commenced. In the mRNA-dependent extract the rate of protein synthesis was less than 5% of that of the controls. Over a period of 120 min in the RNase-treated extracts, the nuclei swelled but did not break down. Thus RNase treatment can effectively destroy endogenous mRNA and block entry into mitosis. When a strongly translated mRNA such as the viral RNA of tobacco mosaic virus was

added to the mRNA dependent extracts, we obtained 50% of the pretreatment level of total protein synthesis but failed to restore the ability of extracts to enter mitosis (data not shown).

We then asked whether the translation of cyclin mRNA was sufficient to allow mRNA-dependent extracts to enter mitosis. Sea urchin cyclin B mRNA was transcribed *in vitro* from a complementary DNA clone (supplied by J. Pines and T. Hunt¹⁷). This mRNA was added to an mRNA-dependent extract at three concentrations. For each reaction, [35 S]methionine was added at the time of RNA addition. The patterns of protein synthesis and nuclear morphology during the course of this experiment are shown in Fig. 3*b* and *c*, respectively. At a cyclin mRNA concentration of 5 $\mu\text{g ml}^{-1}$, cyclin protein accumulated, reaching a peak at 40 min, at which point the nuclei had broken down. By 50 min the nuclei were in telophase and the cyclin band had dramatically decreased in intensity. The cyclin band then increased in intensity to a second maximum at 120 min when the nuclei had once more broken down (Fig. 3*b, c*). Figure 3*d* shows that in mRNA-dependent cell cycle extracts where the cell cycle is driven by the synthesis of sea urchin cyclin, the activities of MPF and H1 kinase increased in prophase, were maintained at high levels during mitosis, and then declined at the end of mitosis. Thus the synthesis of sea urchin cyclin is sufficient to induce both the morphological and biochemical events characteristic of mitosis.

To test whether the rate of cyclin accumulation affected the length of interphase, we added different concentrations of cyclin mRNA to the mRNA-dependent extracts. At 2.5 $\mu\text{g ml}^{-1}$ of cyclin mRNA, the accumulation of cyclin was slightly slower and nuclear envelope breakdown did not occur until 50 min; at 60 min telophase had begun and the cyclin abundance had declined markedly (Fig. 3*b*). At this concentration of mRNA a second round of nuclear envelope breakdown did not occur during the experiment. Finally, at the lowest concentration of mRNA, cyclin accumulated at a slower rate and the nuclei never entered mitosis, nor did the intensity of the labelled cyclin band ever decline (Fig. 3*b*). In experiments that were carried out for longer times, the translation of cyclin mRNA added to mRNA-dependent extracts has induced as many as three rounds of nuclear envelope breakdown. These experiments suggest that cyclin synthesis is sufficient both to induce mitosis and to allow progression from mitosis to the next interphase, and that its rate of accumulation affects the length of interphase.

We have also tested the ability of the *Xenopus* cyclins to induce mitosis. Transcripts of either *Xenopus* B1 or B2 cyclin clones (provided by J. Minshull and T. Hunt) drove the *in vitro* cell cycle (data not shown). Both cyclin proteins increased in abundance during interphase, reached peak levels at nuclear envelope breakdown and had declined by the onset of telophase.

Discussion

We have developed an *in vitro* cell-cycle extract that faithfully reproduces the key features of the cell cycle in early *Xenopus* embryos and performs multiple cell cycles. The endogenous mRNA in such an extract could be destroyed, arresting the cell cycle in interphase and producing an mRNA-dependent extract. In an mRNA-dependent extract, the translation of sea urchin cyclin B or either of two *Xenopus* cyclin B mRNAs was sufficient to drive multiple cell cycles. These results imply that cyclin synthesis can satisfy the protein synthesis requirement both for the activation of MPF and the entry into mitosis, as well as for the degradation of cyclin, inactivation of MPF and progress to the next interphase. The amount of cyclin protein present at mitosis in mRNA-dependent extracts programmed with cyclin mRNA is not substantially different from that translated from the endogenous cyclin mRNA in mock treated extract. This demonstrates that cyclin made in the absence of synthesis of other protein can induce mitosis at about the same level as when the protein is made in untreated extracts.

One limitation to the conclusion that cyclin synthesis is

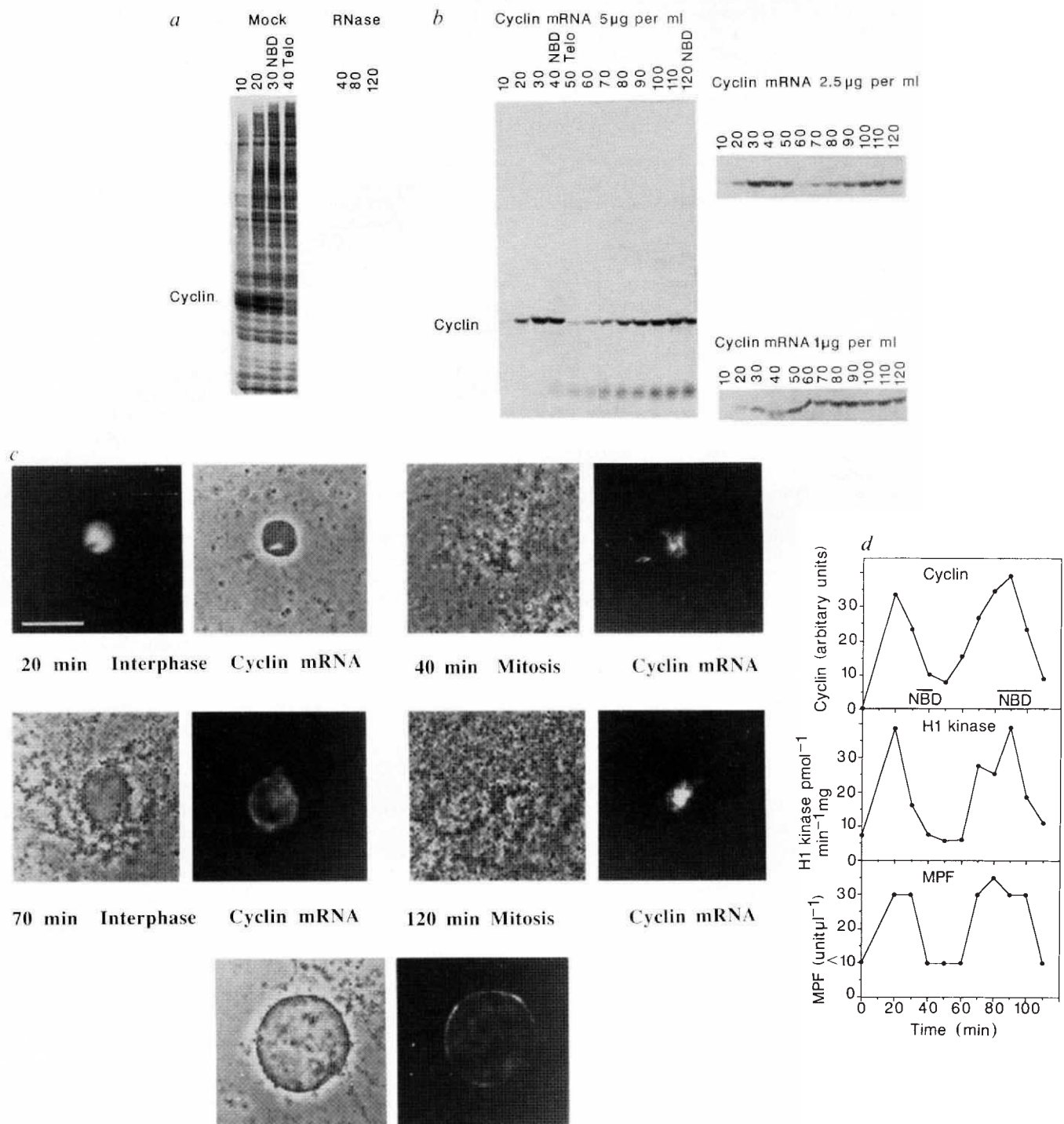


FIG. 3 Cyclin synthesis is sufficient to drive the embryonic cell cycle. *a*, The pattern of protein synthesis in mock treated and RNase-treated extracts. Cell cycle extracts prepared from activated eggs were treated with RNasin (gift of David Drechsel) alone (Mock) or with RNase followed by RNasin (RNase). [^{35}S]methionine was added to the extracts, which were then incubated and samples were removed at the indicated times and run on 10% polyacrylamide gels. The times of nuclear envelope breakdown (NBD) and telophase (Telo) and the position of cyclin are indicated. *b*, The pattern of protein synthesis in mRNA-dependent extracts with different doses of added sea urchin cyclin mRNA. [^{35}S]Methionine and cyclin mRNA were added 10 min after the addition of RNasin and samples were taken for analysis on 10% polyacrylamide gels and for nuclear morphology at the indicated times. The times of nuclear envelope breakdown and telophase are indicated. *c*, Nuclear morphology in the mRNA-dependent extracts with and without added sea urchin cyclin mRNA whose pattern of protein synthesis is shown

in *a* and *b*. Each pair of images consists of a phase-contrast image and a fluorescent image of the DNA-binding dye Hoechst 33342, as described in Fig. 2. Scale bar, 20 μm . *d*, The fluctuations in cyclin abundance and MPF and H1 kinase activity in an mRNA-dependent extract to which 5 $\mu\text{g ml}^{-1}$ of sea urchin cyclin mRNA had been added. The times of nuclear breakdown are indicated (NBD).

METHODS. Cycling extracts were prepared and incubated with a final concentration of 0.25 $\mu\text{g ml}^{-1}$ of boiled pancreatic RNase at 10 $^{\circ}\text{C}$ for 20 min before adding 0.05 volume of a solution of placental RNase inhibitor (RNasin, OD₂₈₀=0.21) and incubating for a further 10 min at 10 $^{\circ}\text{C}$ and then adding calf liver transfer RNA to 50 $\mu\text{g ml}^{-1}$, [^{35}S]methionine to 0.4 mCi ml^{-1} and sea urchin cyclin B mRNA in 100 mM KCl and 1 mM MgCl_2 . The mRNA was transcribed by T7 polymerase from the sea urchin cyclin B cDNA clone as described¹⁷ and then treated with RNase-free DNase to destroy the template DNA. Cyclin abundance was measured by densitometry.

sufficient to drive the cell cycle is that some protein synthesis occurs between activation and the RNase treatment that produces mRNA-dependent extracts. The ability of cyclin synthesis to induce multiple rounds of nuclear envelope breakdown demonstrates that cyclin synthesis is sufficient to induce mitosis in a cell cycle where no other protein synthesis has occurred. Although these experiments show that cyclin synthesis is sufficient to drive later cell cycles, it does not prove that other proteins synthesized in the first cell cycle are not required for the first cell cycle. In the accompanying paper³³ we demonstrate that cyclin protein made in a cell-free translation system can induce entry into mitosis when it is added to an extract where cycloheximide had been used to inhibit protein synthesis before the start of the first mitotic cell cycle. This experiment also shows that the translation of residual endogenous mRNA in the extracts, or of mRNA newly transcribed from the added sperm nuclei, does not supply proteins that are necessary for the induction of mitosis.

The length of the cell cycle increased as the amount of cyclin mRNA was reduced, suggesting that cyclin accumulation has to occur to some critical level to induce entry into mitosis. Thus at least a fraction of the length of interphase in embryonic cell cycles represents the time required to accumulate cyclin to this critical level. This time can be estimated by measuring the time in interphase at which the addition of inhibitors of protein synthesis no longer blocks the occurrence of the next mitosis^{34,35}. This time varies in different embryonic cell cycles, *in vivo*, but is always less than half the length of interphase, suggesting that there are slow steps between the accumulation of cyclin and the activation of MPF and occurrence of mitosis.

The lack of other newly synthesized proteins in the mRNA-dependent extracts strongly suggests that when the cyclin band disappears at the end of mitosis, it does so as a result of proteolysis, rather than as a result of some form of post-translational modification which alters its gel mobility so that it is obscured by other bands. Finally, the stability of the newly synthesized cyclin at the lowest dose of cyclin mRNA shows that the rapid degradation of cyclin is not induced unless it is present in doses sufficient to induce the extract to enter mitosis.

Recent mRNA ablation experiments have shown that cyclin synthesis is necessary for entry into mitosis. In these experiments it was necessary to cleave the mRNAs for both *Xenopus* cyclin B1 and B2 to prevent entry into mitosis¹⁹, confirming the functional redundancy we have seen for these two proteins. The demonstration that cyclin synthesis is both necessary and sufficient for entry into mitosis strongly suggests that cyclin is the only newly synthesized protein required for the induction of mitosis in *Xenopus* embryos.

The ability of cyclin synthesis to induce mitosis suggests an attractive model for the early embryonic cell cycle (Fig. 4a). In this model, cyclin activates the protein kinase activity of p34^{cdc2} that constitutes MPF activity. An opposing activity inactivates this p34^{cdc2} kinase activity. The level of MPF activity is thus controlled by the ratio of the activities of cyclin and the inactivator. Early in interphase cyclin levels are low and the activity of the inactivator is dominant, keeping MPF in its inactive form. Cyclin accumulates during interphase until its activity exceeds that of the inactivator. At this point, MPF activation begins and ultimately triggers a cascade of reactions which lead to the morphological and biochemical events of mitosis. We postulate that MPF induces the degradation of cyclin. Once cyclin has been destroyed, the inactivator is once more dominant, MPF is inactivated and the cell cycle progresses into the next interphase. In this model both interphase and mitosis are unstable states. The instability of interphase is caused by the accumulation of cyclin, whereas that of mitosis is due to the ability of MPF to induce degradation of cyclin. This simple model of the early *Xenopus* cell cycle predicts that cyclin degradation is required to exit from mitosis. The accompanying paper³³ verifies this prediction and examines the mechanism by which cyclin acti-

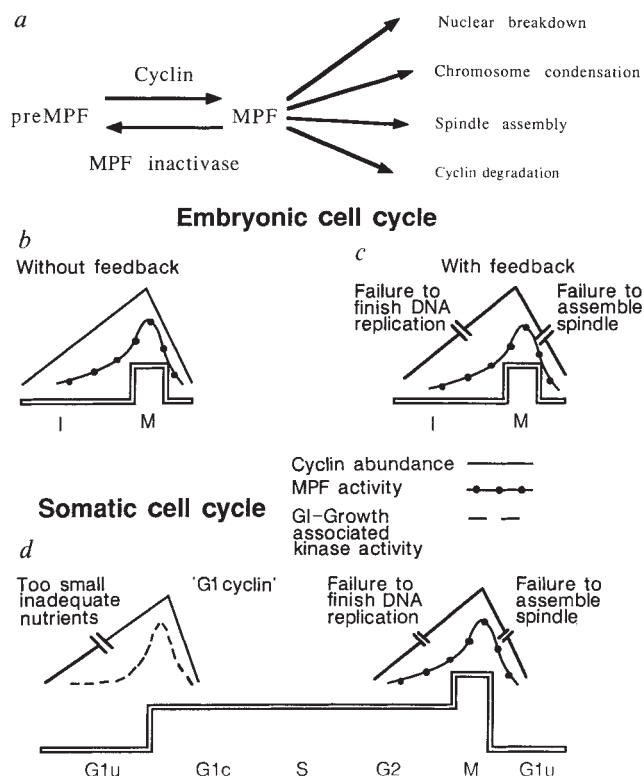


FIG. 4 A model for the cell cycle. *a*, An overview of the proposed reactions controlling passage through the cell cycle. *b*, A diagram of alternation of interphase (I) and mitosis (M), and the levels of cyclin (—) and MPF activity (●—●) in an early embryonic cell cycle without feedback controls. *c*, A diagram of an embryonic cell cycle with feedback controls (II). *d*, A schematic view of a somatic cell cycle showing the role of a G1 cyclin in activating a G1-specific p34^{cdc2} kinase activity (—) to drive the cells from a state where they are not committed to the mitotic cell cycle (Glu), to a state where they are committed to enter DNA synthesis and ultimately progress to mitosis (Glc). See text for further details.

vates MPF.

In this model the accumulation of cyclin initiates a pathway that leads to the post-translational activation of p34^{cdc2} to a form that has MPF activity. During interphase in early *Xenopus* embryos, none of the events in this pathway, except the concentration of cyclin, seems to be regulated, making cyclin the trigger of mitosis. In the more tightly controlled cell cycle of somatic cells, other steps in the pathway that leads to MPF activation, such as cyclin or p34^{cdc2} phosphorylation, may be subject to regulation. One example of this may be the post-cellularization divisions of *Drosophila* embryos. In these cell cycles, cyclin synthesis is required, but it is the accumulation of the gene product of the *string* gene that appears to trigger mitosis³⁶. The *string* gene is the homologue of the *S. pombe* *cdc25* gene, which acts as a dose-dependent activator of the mitosis-inducing function of *cdc2* (ref. 37).

The cyclin-based oscillator in frog extracts lacks feedback controls and will continue to cycle even when processes such as DNA synthesis or spindle assembly are inhibited by aphidicolin and nocodazole (data not shown; see Fig. 4b). The lack of feedback controls in the extract is in good agreement with the previous demonstrations that the oscillations in MPF activity were unaffected by inhibiting DNA synthesis or microtubule polymerization^{3,38,39}. In all somatic cells and in the embryos of many organisms the cell cycle manifests feedback controls that prevent the initiation of one step in the cell cycle until the previous step has been successfully completed⁴⁰. The cyclin-based oscillator may be converted to such a cell cycle by imposing controls on the accumulation and degradation of

cyclin (Fig. 4c). One example is the action of CSF, which appears to stabilize MPF activity by blocking the degradation of cyclin³⁵. In this case, inactivation of CSF and progress into the mitotic cell cycle is made dependent upon an increase in cytoplasmic calcium concentration induced by fertilization. It is easy to imagine that CSF or a related molecule could act in somatic cells to prevent the degradation of cyclin until the spindle has been successfully assembled. In this case the signal that inactivates CSF would be generated by a pathway that monitors some parameter of spindle assembly, such as stable attachment of the kinetochores to microtubules. In sea urchin and clam embryos, microtubule depolymerization greatly increases the length of mitosis and stabilizes cyclin¹⁵. The completion of DNA synthesis could be used to regulate either the accumulation of cyclin, or some post-translational step in the activation of MPF. The latter possibility is suggested by the ability of aphidicolin to block sea urchin embryos in interphase, even though cyclin accumulates to very high levels (T. Hunt, personal communication).

In early embryos, regulation of the cell cycle appears to be limited to the control of the entry into and the exit from mitosis. Somatic cells possess an additional control point located in G1, where cells can either become committed to passage through the cell cycle or remain in a resting state. This commitment

point has been named 'Start' in studies on yeasts and the restriction point in mammalian tissue culture cells^{40,41}. Three lines of evidence fuel the speculation that passage through Start requires the accumulation of a molecule related to cyclin that induces a particular spectrum of p34^{cdc2} kinase activity (Fig. 4d). First, experiments on tissue culture cells suggest that the accumulation of an unstable protein to some critical level is required to pass the restriction point⁴². Second, the *cdc2* gene of *S. pombe* is required at Start as well as for the induction of mitosis⁴³. The Start function of *cdc2* is independent of the genes that regulate its mitotic function, *cdc25*, *weel* and *cdc13*, the *S. pombe* B-type cyclin, suggesting both that the substrate specificity and kinase activity of p34^{cdc2} at Start and the induction of mitosis are different, and that a second group of genes that has not yet been identified exists to modulate the activity of p34^{cdc2} at Start⁸. Third, homologues of both cyclin and p34^{cdc2} have been identified among mutants which affect the ability of mating pheromones to arrest the cell cycle of *S. cerevisiae* at Start^{44,45} (W. Courchesne and J. Thorner, personal communication). These lines of evidence strongly suggest that the interaction between members of the cyclin and p34^{cdc2} families will play a key part in the regulation of Start as well as in the induction of mitosis. □

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The role of cyclin synthesis and degradation in the control of maturation promoting factor activity

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We show that cyclin plays a pivotal role in the control of mitosis. A proteolysis-resistant mutant of cyclin prevents the inactivation of maturation promoting factor and the exit from mitosis both *in vivo* and *in vitro*. We have used a fractionated extract to study the activation of MPF by added cyclin protein.

THE activation of a protein complex called maturation promoting factor (MPF) induces cells to enter mitosis and meiosis. In the accompanying paper¹ we show that cyclin is the only newly synthesized protein required to induce MPF activity and the entry into mitosis in the cell cycle of early *Xenopus* embryos. This finding implies that in early embryonic cell cycles, none of the other components of MPF needs to be synthesized in each cell cycle, and that all steps in MPF activation and inactivation other than cyclin accumulation are post-translational events. Here we investigate the role of cyclin synthesis and degradation

in the control of MPF activity.

Two lines of evidence suggest that cyclin degradation may be required for the inactivation of MPF and exit from mitosis and meiosis: (1) Extending the duration of the mitotic state with pharmacological agents increases the stability of cyclin, and (2) high concentrations of protease inhibitors arrest starfish oocytes in meiosis I (ref. 2) and stabilize cyclin (T. Hunt, personal communication).

The recent purification of MPF has led to a more detailed understanding of its molecular nature. MPF appears to be identical to the growth-associated histone H1 kinase and purified preparations of both these activities contain p34^{cdc2}, the homologue of the product of the *cdc2* gene of *Schizosaccharomy-*

ces pombe, whose activity is required for entry into mitosis³⁻⁵.

The biochemistry of MPF activation has been studied in immature oocytes. In these cells MPF activation and meiosis I can be induced in the absence of protein synthesis, implying that such oocytes contain an inactive form of MPF, which has been termed pre MPF^{6,7}. In *Xenopus* oocytes, the post-translational conversion of preMPF into MPF requires ATP and small quantities of MPF. The role of MPF in this reaction appears to be to antagonize an inhibitory factor named INH, which inhibits the conversion of preMPF into MPF⁷.

To examine the role of cyclin proteolysis in the exit from mitosis and meiosis we prepared cell-cycle extracts that preserve the cytosolic factor (CSF)-mediated metaphase arrest of unfertilized eggs. Cyclin is stable in CSF-arrested extracts but is rapidly degraded when these extracts are induced to re-enter the cell cycle by the inactivation of CSF. A mutant cyclin that retains the ability to induce mitosis but is resistant to proteolysis arrests the cell cycle in metaphase even when CSF activity is

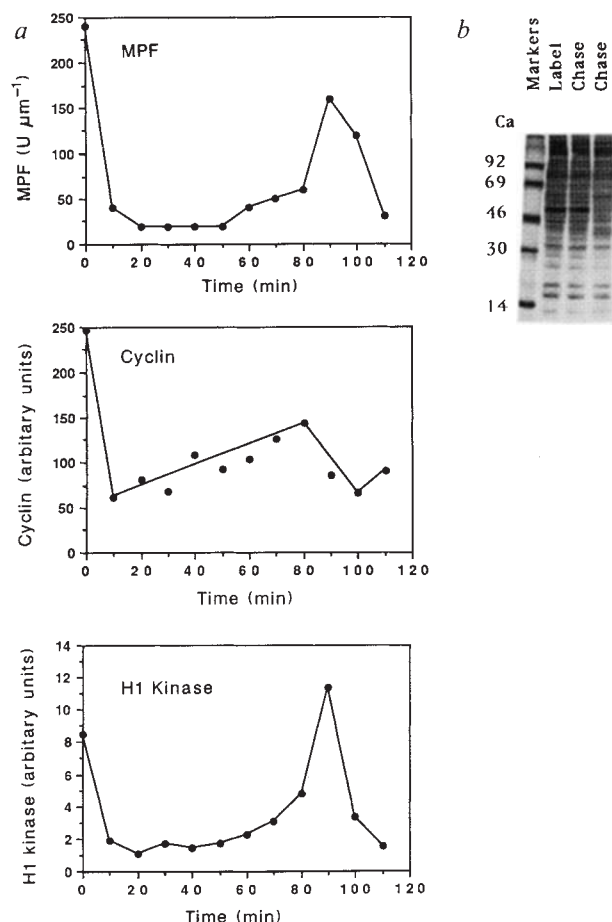


Fig. 1 Behaviour of CSF-arrested extracts. *a*, The abundance of cyclin and the activities of MPF and H1 kinase in a CSF-arrested extract after incubation with [³⁵S]methionine for 30 min before the addition of calcium to 0.2 mM at time zero. *b*, The stability of cyclin in CSF-arrested extracts. 100 $\mu\text{g ml}^{-1}$ of cycloheximide was added to a CSF-arrested extract which had been incubated with [³⁵S]methionine for 60 min (Label) and one half of this extract had CaCl₂ added to a final concentration of 0.4 mM. Both calcium-treated and control halves of the extract were subjected to a 40-minute chase (Chase).

METHODS. CSF-arrested extracts were prepared as follows. *Xenopus* eggs were squeezed into MMR (ref. 1), dejellied with cysteine and washed four times with XB (ref. 1) and then twice with XB containing the following additions: 5 mM KEGTA, pH 7.7, 1 mM MgCl₂, 10 $\mu\text{g ml}^{-1}$ each of leupeptin, pepstatin and chymostatin. The eggs were then transferred to a 5 ml centrifuge tube containing 1 ml XB with the additions described above plus 100 $\mu\text{g ml}^{-1}$ cytochalasin B. Versilube F-50 oil was used to remove excess buffer for extracts made from activated eggs¹ and the packed eggs were then spun for 10 min at 15,000g at 15°C. The cytoplasmic fraction was made 10 mM in creatine phosphate and 10 $\mu\text{g ml}^{-1}$ each in leupeptin, chymostatin and pepstatin and spun for 15 min at 15,000g at 2°C, then sperm nuclei were added to a final concentration of 10⁵ per ml. MPF and H1 kinase activities and cyclin abundance were measured as described¹.

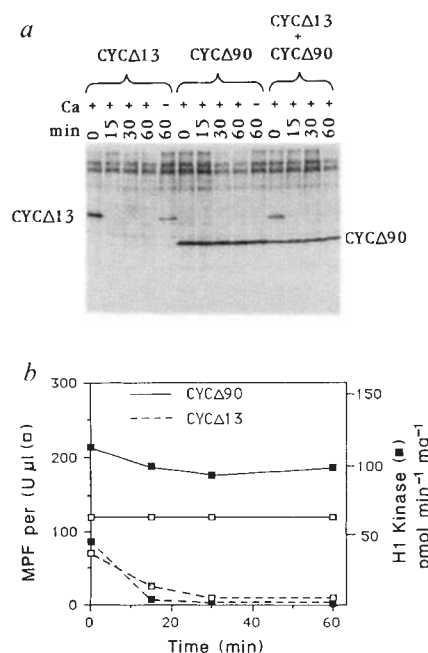


FIG. 2 A truncated cyclin is resistant to degradation. *a*, An autoradiograph of a 12.5% polyacrylamide gel of [³⁵S]methionine-labelled CSF-arrested extracts containing the mRNAs encoding CYCΔ13, CYCΔ90 or both mRNAs together, at the indicated times after the addition of calcium. *b*, The levels of MPF and H1 kinase activity during calcium-induced release from CSF arrest in extracts containing CYCΔ13 or CYCΔ90 whose pattern of labelled proteins is shown in *a*. The mRNAs were added to a CSF-arrested extract, containing [³⁵S]methionine at 0.4 mCi ml⁻¹, at a final concentration of about 10 $\mu\text{g ml}^{-1}$. The extract was incubated at 23°C for 40 min before addition of cycloheximide to 100 $\mu\text{g ml}^{-1}$. The extract was then split into two portions, CaCl₂ was added to one portion to a concentration of 0.4 mM, and samples were taken at the indicated times and diluted in sample buffer for analysis on gels, fixed to visualize their nuclear morphology and processed for MPF, cyclin, and H1 kinase assays as described.

METHODS. The clones CYCΔ13 and CYCΔ90 were constructed as follows. A 1.3-kb *NcoI*-*HindIII* fragment from the sea urchin B cyclin complementary DNA containing the coding sequence of the sea urchin cyclin B gene, minus the first 13 amino acids, was ligated to the vector pSPBP4 (ref. 19; a gift from William Hansen) that had been cleaved with *NcoI* and *XbaI*, after filling in the *HindIII* and *XbaI* ends with T4 DNA polymerase. The resulting plasmid, A400p27, has the cyclin gene out of frame with the ATG provided by pSPBP4. CYCΔ13 was made by cleaving A400p27 with *NcoI*, filling in the sticky ends with T4 DNA polymerase and ligating with T4 DNA ligase to place the cyclin gene in frame with the ATG. CYCΔ90 was made by cleaving A400p27 with *BglII* and *NcoI*, filling in the sticky ends with T4 DNA polymerase and ligating with T4 DNA ligase. Capped CYCΔ13 and CYCΔ90 mRNAs were made by transcribing CYCΔ13 or CYCΔ90 DNA with SP6 RNA polymerase, including GpppG or m⁷GpppG in the transcription reaction²⁰.

absent. Finally, we have started to define the biochemical role of cyclin protein by preparing fractionated extracts where the addition of exogenous cyclin induces the activation of MPF.

CSF-arrested extracts

We have used CSF arrested extracts to investigate the fate of cyclin during a physiological metaphase arrest and during the exit from meiosis. Lohka and Masui showed that extracts made from unfertilized *Xenopus* eggs in the presence of EGTA retain the CSF-mediated arrest in meiotic metaphase, directly convert added sperm nuclei into condensed chromosomes on meiotic spindles and can be induced to progress to interphase by the addition of calcium⁸. Thus calcium can release the cell cycle from CSF arrest *in vitro* as well as *in vivo*. We prepared similar, but more concentrated, extracts where the addition of as little as 0.2 mM calcium induced the inactivation of CSF, spindle dissolution, chromosome decondensation and the formation of interphase nuclei within 30 min.

Figure 1a shows cyclin levels, MPF and H1 kinase activity in CSF-arrested extracts and in CSF-arrested extracts induced to re-enter the cell cycle by calcium treatment. Before the addition of calcium the levels of cyclin, MPF and H1 kinase activity were all high. Within 10 min of calcium addition, cyclin levels had dramatically decreased, as had those of MPF and H1 kinase activity. During interphase the levels of cyclin increased continuously but H1 kinase and MPF activity appeared suddenly at 90 min corresponding to the time of nuclear envelope breakdown, showing that H1 kinase activity is proportional to MPF activity but not to cyclin abundance. By 100 min the nuclei were in telophase and MPF and H1 kinase activity had decreased. A control portion of the same extract, without added calcium, maintained high levels of cyclin, MPF and H1 kinase activity for more than 80 min (data not shown), suggesting that cyclin degradation is induced by the destruction of CSF.

We examined the stability of cyclin during CSF arrest by performing a pulse-chase experiment. We added [³⁵S] methionine to a CSF-arrested extract for 20 min and then added cycloheximide to prevent further protein synthesis. Figure 1b shows that the labelled cyclin was completely stable during a further 40 min incubation. In contrast, when calcium was added immediately after the cycloheximide, the cyclin bands disappeared although the other labelled proteins were completely stable, suggesting that one of the functions of CSF is to prevent the cyclin degradation that normally occurs when MPF is active. CSF-arrested extracts that were induced to enter interphase, after protein synthesis had been inhibited by cycloheximide, never entered mitosis, confirming the protein synthesis requirement for the induction of mitosis.

Proteolysis-resistant cyclin

If the domain of cyclin that is responsible for the activation of MPF activity is separable from the one that directs cyclin proteolysis we could ask directly whether cyclin proteolysis is required for the exit from mitosis. We constructed two plasmids CYCΔ13 and CYCΔ90 that contain deletions of the N-terminal 13 and 90 amino acids of the sea urchin cyclin B, respectively, and which can be transcribed by SP6 RNA polymerase to yield highly translatable messenger RNA. We refer to the proteins encoded by these plasmids as the CYCΔ13 and CYCΔ90 cyclins. We have used the CYCΔ13 cyclin as a control in the following experiments because the CYCΔ13 mRNA is much more efficiently translated than full length sea urchin cyclin mRNA used in the preceding paper¹. The biological properties of the full-length and CYCΔ13 proteins appear to be identical.

We examined the proteolysis of these truncated cyclins in CSF arrested extracts and during the exit from metaphase after the calcium-induced inactivation of CSF. The synthetic cyclin mRNAs were translated for 40 min in a CSF-arrested *Xenopus* cell-cycle extract containing [³⁵S] methionine before cycloheximide was added to prevent further protein synthesis. Cal-

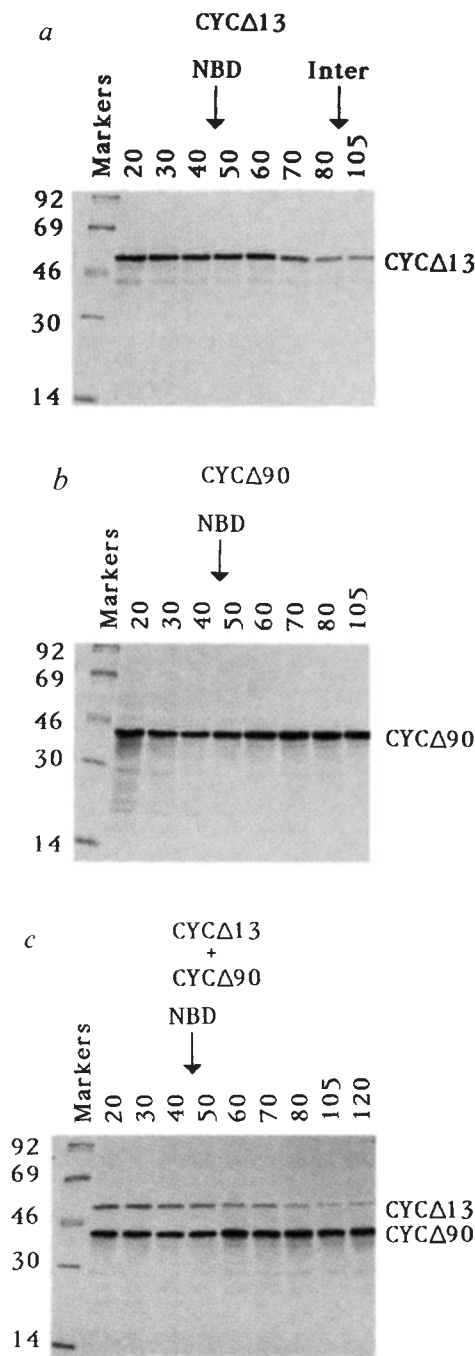


FIG. 3 Induction of mitosis by cyclin made in reticulocyte lysate. *a*, Autoradiograph of a 12.5% polyacrylamide gel showing samples taken at the indicated times after the addition of 10 μ l reticulocyte lysate containing approximately 50 nM [³⁵S]methionine-labelled CYCΔ13 cyclin to 20 μ l a CSF-arrested extract that had been induced to enter interphase after the addition of cycloheximide. The times of nuclear breakdown (NBD), the reappearance of interphase nuclei (Inter), the position of the CYCΔ13 cyclin and the molecular weight markers (in thousands) are indicated. *b*, Addition of reticulocyte lysate containing ~50 nM CYCΔ90 cyclin; other details as in *a*. *c*, Addition of reticulocyte lysate containing ~15 nM CYCΔ13 and ~35 nM CYCΔ90; other details as in *a*.

METHODS. Sperm nuclei were added to a CSF-arrested extract which was then made 20 μ g ml⁻¹ in cycloheximide before the addition of CaCl₂ to 0.4 mM to induce progression into interphase. Twenty minutes after the addition of calcium, 0.5 volumes of reticulocyte lysate containing translated cyclin protein was added. CYCΔ13 or CYCΔ90 mRNA was translated in message-dependent rabbit reticulocyte lysate²¹ and the amount of synthesized protein was calculated from the amount of incorporated methionine, the cold methionine pool size in the reticulocyte lysate and the number of methionines in CYCΔ13 or CYCΔ90.

cium was then added to the extract to induce the inactivation of CSF, destruction of endogenous cyclin and entry into interphase. After calcium addition, we monitored the pattern of labelled proteins, nuclear morphology, MPF and H1 kinase activity. Extracts containing the CYCΔ13 cyclin entered interphase rapidly as judged by the formation of interphase nuclei (data not shown) and the loss of MPF and H1 kinase activity (Fig. 2*b*). The CYCΔ13 cyclin, but not other labelled non-cyclin proteins, was degraded (Fig. 2*a*). In a control extract to which calcium was not added the levels of the CYCΔ13 cyclin, MPF and H1 kinase decreased less than twofold during a 60-min incubation in the presence of cycloheximide. Thus deletion of the first 13 amino acids of cyclin does not alter its susceptibility to the cyclin-specific proteolysis that is induced in the mitotic state or the ability of CSF to protect it from such degradation. In contrast, in extracts containing the CYCΔ90 cyclin, the addition of calcium did not induce the degradation of CYCΔ90, the exit from metaphase, or decrease the activity of MPF and H1 kinase (Fig. 2*a,b*). These results are consistent with the hypothesis that CYCΔ90 is resistant to proteolysis and that cyclin degradation is required for exit from CSF arrest. As a further test we added calcium to a CSF arrested extract containing both CYCΔ13 and CYCΔ90 cyclins (Fig. 2*a*). This extract remained in metaphase and the CYCΔ13 cyclin was degraded but the CYCΔ90 cyclin was not, demonstrating that the CYCΔ90 cyclin does not inhibit the inactivation of CSF, or block the proteolysis of intact cyclin molecules in some other way.

We then tested the ability of the deleted cyclins to induce mitosis by adding protein translated in reticulocyte lysate to a CSF-arrested extract that had been induced to enter interphase after the addition of cycloheximide. The addition of reticulocyte

lysate containing the translation products of an irrelevant mRNA, such as tobacco mosaic virus (TMV) RNA did not induce mitosis and the translation products were stable in the extract. Figure 3*a* shows that the addition of reticulocyte lysate containing the CYCΔ13 cyclin induced nuclear envelope breakdown after 40–50 min. The cyclin was then degraded and the extract returned to interphase. (Smaller doses of the CYCΔ13 cyclin failed to induce mitosis and the added cyclin was stable for at least two hours (data not shown).) In contrast, the addition of the CYCΔ90 cyclin induced nuclear envelope breakdown, but the cyclin was stable and the extract remained in mitosis for the duration of the experiment (Fig. 3*b*). When CYCΔ13 and CYCΔ90 cyclins were added together the extract entered mitosis and induced the degradation of the CYCΔ13 cyclin, but failed to return to interphase (Fig. 3*c*). These experiments demonstrate that the deletion of the N-terminal 90 amino acids of the sea urchin cyclin does not affect its ability to induce mitosis but renders it resistant to proteolysis and strongly suggest that cyclin degradation is required for the exit from mitosis as well as meiosis.

The presence of the CYCΔ90 cyclin can also arrest the cell cycle *in vivo*. We injected unfertilized eggs with mRNA and allowed the mRNA to translate for two hours before activating the eggs by injecting calcium and recording their behaviour by time-lapse video microscopy. Eggs that received CYCΔ13 RNA, CYCΔ90 RNA, TMV RNA or no RNA showed the normal cortical activation reaction in which the pigment contracts towards the animal pole and the egg rounds up after activation (Fig. 4*b*). In the TMV RNA and control eggs, after the initial contraction the pigment relaxed, the cortex became less stiff and the egg spread out under the force of gravity. Approximately

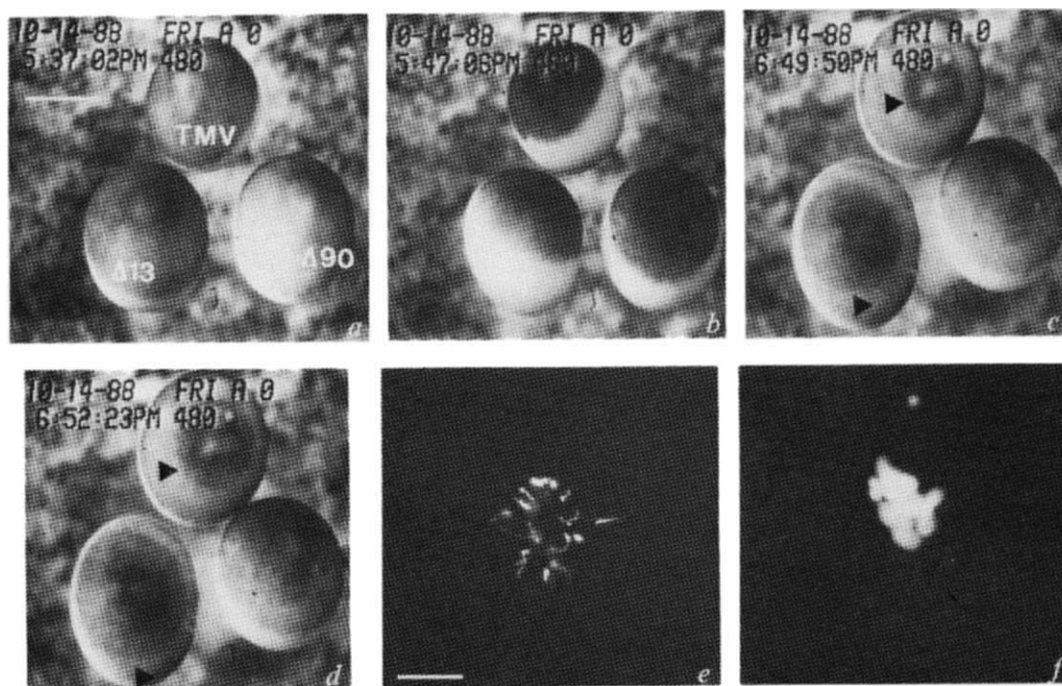


FIG. 4 CYCΔ90 cyclin arrests the embryonic cell cycle *in vivo*. *a–d*, Photographs of a time-lapse video recording of eggs that had been injected, in non-activating medium (100 mM KCl, 0.5 mM MgCl₂, 0.1 mM CaCl₂, 25 mM KPIPES, pH 6.5) with 50 nl of a 1 mg ml⁻¹ solution of CYCΔ13 (Δ13), CYCΔ90 (Δ90) or tobacco mosaic virus (TMV) RNA two hours before they were activated by injection of 20 nl MMR. The four images show the eggs: *a*, before activation (the pigmentation of all three eggs is identical, but the egg injected with CYCΔ90 mRNA was more strongly illuminated at this time); *b*, 5 min after activation; *c* and *d*, at two points during the passage of the first surface contraction wave across the eggs injected with CYCΔ13 or TMV RNA. The surface contraction waves (▶) are visible as rings of lighter

pigmentation that originate at the animal pole (the centre of the visible hemisphere of the egg in this figure) and that move across the surface of the egg. There is no visible surface contraction wave in the egg injected with CYCΔ90 mRNA. (Scale bar, 1 mm). *e*, The single set of mitotic chromosomes from a fertilized egg that had been injected with 50 nl of a 1 mg ml⁻¹ solution of CYCΔ90 mRNA. The egg was squashed and fixed in the presence of Hoechst 33342 (ref. 1) at 150 min after fertilization. (Scale bar, 20 μm). *f*, One of fourteen telophase nuclei in a fertilized egg that had been injected with 50 nl of a 1 mg ml⁻¹ solution of TMV RNA at 20 min after fertilization. The egg was squashed and fixed at 155 min after fertilization. Same magnification as in *e*.

50 min after activation these eggs rounded up, as the level of MPF rose. Shortly afterwards a surface contraction wave (SCW) propagated across the surface of the egg (Fig. 4c, d). Strong circumstantial evidence supports the notion that the SCW is induced by the decline in MPF activity that follows each mitosis^{6,9,10}. At 25°C the first SCW occurs about 60 min after activation and subsequent ones occur at 30-min intervals. In eggs that had been injected with CYCA13 mRNA (Fig. 4c, d) the egg also undergoes normal SCWs, but the interval between the later SCWs was shorter than that in uninjected or TMV injected controls, suggesting that the translation of cyclin at high levels may shorten the length of the cell cycle. Although eggs injected with CYCA90 mRNA showed a normal cortical reaction upon activation (Fig. 4b), the cortex never lost its stiffness, the eggs never spread out and no SCWs were seen (Fig. 4c, d). Thus the presence of the deleted cyclin *in vivo* prevents the escape from CSF mediated meiotic arrest. The occurrence of the normal cortical contraction at activation and the elevation of the eggs' vitelline membrane in eggs arrested in metaphase with the deleted cyclin demonstrates that these events are dependent on the increased levels of intracellular calcium induced by activation rather than by the decline in MPF that this calcium spike normally induces.

To show that the deleted cyclin can arrest cells in mitosis as well as in meiosis we injected fertilized eggs with CYCA90 or TMV RNA during the first cell cycle and observed them for two and a half hours. The eggs injected with CYCA90 mRNA failed to cleave during this period. To visualize the nuclei we squashed eggs and observed their DNA using a fluorescent DNA-binding dye. Figure 4e shows the single set of condensed chromosomes found in an embryo that had been injected with CYCA90 mRNA, whereas Fig. 4f shows one of 14 telophase nuclei found in a single control embryo that had been injected with TMV RNA. We conclude that the presence of the deleted cyclin encoded by CYCA90 can arrest the embryonic cell cycle in mitosis *in vivo* as well as *in vitro*, demonstrating that cyclin degradation is required for the exit from both meiosis and mitosis.

Cyclin and MPF activation

We have started to investigate the biochemistry of cyclin. The demonstration that cyclin synthesis is required to drive the embryonic cell cycle into mitosis and that cyclin degradation is required for the exit from mitosis implies that cyclin has a role in the activation and maintenance of MPF activity. Possible mechanisms by which cyclin could activate p34^{cdc2} to yield MPF activity include: binding to p34^{cdc2} to form the active MPF complex; stimulating the post-translational activation of p34^{cdc2}; or acting to inhibit an inactivator of the MPF activity of p34^{cdc2}.

In the oocyte pre-MPF can be activated to MPF post-translationally, by the removal of an inhibitor fraction called INH, and incubation of the partially purified fraction with ATP or ATP γ S (a more potent activator of MPF than ATP⁷, presumably because the thiophosphate group that it introduces into proteins is resistant to the action of phosphatases¹¹). We first asked whether removal of INH also leads to the post-translational activation of MPF from CSF-arrested extracts that were activated and returned to an interphase state, that is, whether protein synthesis, or more specifically cyclin synthesis, is required to activate MPF when INH is removed. We prepared an extract deficient in cyclin by activating CSF-arrested extracts in the presence of cycloheximide; as a control, the same CSF-arrested extract was activated in the absence of cycloheximide. Activation with calcium destroys the endogenous cyclin. Samples were taken during the ensuing interphase from both extracts. At various times samples of the extracts were fractionated by precipitation with 33% ammonium sulphate which precipitates MPF and pre MPF and separates them from INH, which precipitates in the 45–55% ammonium sulphate fraction^{7,12} (M. S. and T. Lee unpublished data). The 0–33% fractions were incubated with ATP γ S and assayed for MPF activity by injection into

cycloheximide-arrested oocytes.

The results of this experiment are shown in Fig. 5a. In the extract where protein synthesis was allowed to proceed, the ability of ATP γ S to convert preMPF into active MPF gradually appeared during interphase. But, none of the fractions prepared from the cycloheximide treated extract could be activated by ATP γ S to yield active MPF, demonstrating that protein synthesis is required to produce an activity required for the activation of MPF. Because the only newly synthesized protein required for the appearance of MPF activity in unfractionated extracts is cyclin, this result strongly implies that cyclin is directly required for generating MPF, rather than inhibiting INH.

We then tested directly the ability of exogenous cyclin protein to stimulate the formation of MPF when added to a fraction deficient in cyclin. We prepared a cyclin-deficient extract by activating a CSF-arrested extract in the presence of cycloheximide, as in Fig. 5a. From this extract we prepared a 0–33%

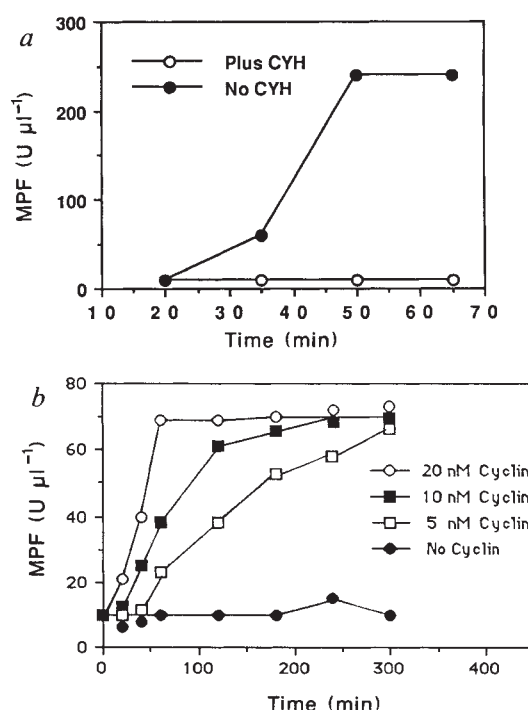


FIG. 5 Cyclin activates MPF *in vitro*. *a*, The time course of the appearance of MPF activity in a CSF-arrested extract that had been induced to progress into interphase in the presence (○—○) or (●—●) absence of cycloheximide. *b*, Kinetics of the induction of MPF activity after addition of cyclin protein. METHODS. *a*, One aliquot of a CSF-arrested extract was made up to 60 μg ml⁻¹ in cycloheximide and the other was left untreated. Both portions were induced to progress into interphase by adding CaCl₂ to 0.3 mM. At the indicated times 60 μl samples were taken, chilled, diluted with 200 μl EB (composition in ref. 1) containing 5 mM dithiothreitol, 0.25 mM PMSF and 10 μg ml⁻¹ each of leupeptin, pepstatin and chymostatin and spun for 20 min at 100,000 r.p.m. in a Beckman Airfuge. The supernatant was precipitated by addition of ammonium sulphate to 33% saturation. The ammonium sulphate pellet was resuspended in 15 μl EB plus protease inhibitors and dialysed for 1 h against two changes of EB plus 5 mM dithiothreitol. (Large preparations of this fraction were made by scaling up this procedure using CSF-arrested extracts to which cycloheximide had been added. After dialysis the fraction was frozen in liquid nitrogen and stored in small aliquots at -70 °C.) The dialysed material was made 9 mM in ATP- γ S and incubated for 75 min at 20 °C and then assayed for MPF activity by microinjection into cycloheximide-treated immature *Xenopus* oocytes⁴. Incubation of the fraction without ATP- γ S did not activate MPF (data not shown). *b*, Each reaction contained 0.5 volumes of the activated CSF fraction described in *a*, 0.1 volumes 9 mM ATP- γ S and 0.4 volumes of reticulocyte lysate containing different amounts of CYCA90 cyclin that had been previously synthesized *in vitro* to yield the indicated final cyclin concentrations. After generating the cyclin protein in the reticulocyte lysate, no protein synthesis occurred in any further reactions.

ammonium sulphate fraction and added reticulocyte lysate containing previously synthesized cyclin protein (CYCΔ90). The mixture was incubated with ATPγS and assayed at various times for MPF activity. In the complete reaction, MPF activity and H1 kinase activity were generated showing that added cyclin and ATPγS can activate MPF. The omission of the *Xenopus* egg fraction or ATPγS, or the substitution of reticulocyte lysate containing TMV translation products completely blocked the ability to generate active MPF. The activation of MPF is not a unique property of the proteolysis-resistant cyclin; the addition of the CYCΔ13 cyclin also induced MPF activation (data not shown).

The addition of different amounts of the CYCΔ90 cyclin to the reaction affected the rate at which MPF activity was generated but not its final level (Fig. 5b). This suggests that if cyclin is a component of activated MPF the amount of one of the other components found in the 33% ammonium sulphate fraction must be limiting, even at the lowest doses of added cyclin. Alternatively, cyclin may be acting to stimulate the activation of MPF but may not be a tightly bound component of the active MPF complex.

Discussion

We have attempted to verify the prediction, made in the accompanying paper, that cyclin degradation is required for the exit from mitosis. A truncated form of the sea urchin cyclin B, CYCΔ90, is resistant to proteolysis and its presence arrests the cell cycle in mitosis. The simplest interpretation of this finding is that the deletion of the N-terminal 90 amino acids of cyclin removes sequences required for the degradation of cyclin without affecting other properties of the molecule. Alternatively, the N-terminus of cyclin might play some active role in the inactivation of MPF, such as guiding MPF to a site where it is inactivated, and cyclin degradation could be a secondary event, which occurred as a consequence of the inactivation of MPF. If this were the case, the addition of a large excess of the CYCΔ13 cyclin (which behaves as a full-length cyclin) ought to overcome the ability of the CYCΔ90 cyclin to arrest extracts in metaphase. We have found that the ability of the CYCΔ90 cyclin to arrest extracts in mitosis cannot be overcome by a tenfold excess of CYCΔ13, suggesting that CYCΔ90 arrests extracts in mitosis because it is resistant to proteolysis and not because it lacks some function required directly for MPF inactivation. A possibility which is more difficult to exclude is that the ability of cyclin to maintain MPF activity is abrogated by some modification of cyclin, that CYCΔ90 is not a substrate for this modification and that cyclin degradation is a consequence of the inactivation of MPF. Even if it were possible to arrest extracts or cells in mitosis by overproducing a wild-type cyclin, it would be impossible to demonstrate that the arrest was a consequence of saturating cyclin proteolysis, rather than some inhibitory cyclin modification.

At present we understand very little about cyclin proteolysis. Although it can be inhibited by a number of protease inhibitors both in starfish (T. Hunt, personal communication) and *Xenopus* (our unpublished data), the high inhibitor concentrations that are required also inhibit protein synthesis and nuclear decondensation, suggesting that the inhibition of cyclin degradation in these experiments may be non-specific. Even in experiments where cyclin is the only labelled protein, we have failed to see intermediates in degradation, suggesting that once degradation has begun it proceeds rapidly to completion. The stability of CYCΔ90 suggests that in some way the N-terminal region is required for cyclin proteolysis. But without an extensive series of mutants in this region it is impossible to draw any more specific inference. At least one site required for cyclin phosphorylation has been localized to about amino-acid 70 in the sea urchin cyclin sequence (S. Mackie and T. Hunt, personal communication) and it has been shown that inhibitors of protein phosphorylation can block the degradation of cyclin in *Xenopus*

egg extracts (M. Felix and E. Karsenti, personal communication). We speculate that the phosphorylation of this sequence is required for cyclin degradation.

We have shown that exogenous cyclin protein can activate MPF in a fractionated cell-cycle extract and presented evidence that, in this reaction, the exogenous cyclin affects the rate but not the final level of MPF activity. These experiments were performed on a fraction that was prepared from CSF-arrested extracts that had been treated with cycloheximide before activation by added calcium. We have shown that under these conditions recently synthesized frog cyclin is largely destroyed. In the absence of antisera to all of the frog cyclins we cannot demonstrate that all of the cyclin in the eggs is destroyed and must therefore admit that there may be some *Xenopus* cyclin in this fraction. Nevertheless, it is clear that the addition of exogenous cyclin is required for the generation of MPF activity and that the final level of MPF is independent of the concentration of cyclin. The simplest interpretation is that either p34^{cdc2} or some other component is limiting or that each cyclin molecule can activate more than one p34^{cdc2} molecule.

Evidence that cyclin need not be a stably bound component of MPF comes from studies of purified H1 kinase, which has high levels of MPF activity, but is reported to contain only p34^{cdc2} (ref. 13). We wish to emphasize that the existence of forms of MPF that lack cyclin may be properties of *in vitro* systems, where inactivating components have been removed. In the natural system, cyclin may be required to stabilize MPF activity from inactivation. We suggest that much of the variability of the polypeptide components of active MPF or H1 kinase preparations may reflect the complex physiological regulation of these activities, and, at least in the case of MPF, the extremely complex system in which it is assayed. For instance, the particular substrate specificity of the protein kinase that is assayed as MPF may be manifested by p34^{cdc2} molecules carrying a particular constellation of post-translational modifications, even when p34^{cdc2} is not associated with other proteins. But, the attainment or maintenance of this phosphorylation state may require the association of other proteins, including, but not necessarily limited to, the cyclins. If the stability of these associations and particular modification states of p34^{cdc2} varied between different organisms and different experimental conditions, then it would be possible to produce a wide variety of protein complexes that had MPF activity.

Evidence that cyclin interacts directly with p34^{cdc2} comes from the genetic demonstration of allele-specific interactions between *cdc2* and *cdc13*, the cyclin homologue in *S. pombe* (refs 14–17), and precipitation of cyclin with anti-p34^{cdc2} antibodies and vice versa¹⁸. Perhaps cyclin may act in different ways both upstream and downstream of p34^{cdc2}. Cyclin accumulation may be required directly or indirectly to activate p34^{cdc2}, but may then itself be phosphorylated by the activated p34^{cdc2} and act directly or indirectly in the pathways by which p34^{cdc2} kinase activity produces the mitotic state. Recent evidence in *S. pombe*, supports this idea. Deletion of the *S. pombe* cyclin homologue, *cdc13*, prevents the entry into mitosis and the activation of the p34^{cdc2} kinase activity, but the missense mutation *cdc13-117* arrests cells in an aberrant mitosis where p34^{cdc2} kinase activity is hyperactivated, the chromosomes are hypercondensed, but no mitotic microtubule structures are present (P. Nurse, personal communication).

It is clear that cyclin has an important role in the activation and maintenance of the particular set of post-translational modifications of p34^{cdc2} that constitute MPF activity. However, the molecular details of how cyclin participates in the post-translational activation of p34^{cdc2}, the nature of other proteins involved in the activation and inactivation reactions, the physiological substrates of MPF and the mechanism of cyclin degradation remain largely unclear. The next few years seem certain to bring a considerable advance in our understanding of how the entry into and the exit from mitosis are controlled. □

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A new class of extragalactic radio sources with one-sided structure?

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EXTRAGALACTIC radio sources with extended emission on only one side of the active galaxy usually have very dominant radio cores^{1–5}, large misalignments between the structure inferred from Very Long Baseline Interferometry and that on the arcsecond scale^{1,6,7}, and often evidence of ‘blazar’ characteristics and superluminal motion in the nucleus⁸. These properties are usually attributed to relativistic motion of nuclear jets in sources inclined at small angles to the line of sight. From a large survey of possible one-sided sources made with both MERLIN and the Very Large Array, we have been able to identify a class of one-sided sources that are dominated by their extended emission while the lower limits on their degree of asymmetry, inferred from our MERLIN observations, are amongst the highest known. These weak-cored one-sided sources appear to be inconsistent with the ‘unified scheme’^{9–15}, which attempts to explain core-dominated sources as

being the relativistically beamed counterparts of the lobe-dominated ones. We discuss possible explanations for this class of sources and suggest tests to distinguish between the different alternatives.

The vast majority of high-luminosity ($\geq 2 \times 10^{25}$ W Hz⁻¹ sr⁻¹ at 178 MHz extragalactic radio sources, especially when selected at a low frequency (≤ 1 GHz), have reasonably symmetric outer lobes on opposite sides of the nucleus; the median value of the distribution of the peak brightness ratio is ~ 3 . However, a small but significant fraction of these sources seem to have radio emission on only one side of the nucleus. These one-sided sources, also referred to as being of the D2 type¹⁶ or C type¹, have very prominent radio cores, and many of their observed properties are consistent with the predictions of ‘unified schemes’. The one-sidedness of some of the core-dominated sources must then be due largely to relativistic beaming of the extended emission so that the approaching component is Doppler-boosted and the receding one is diminished^{10,11}. The brightness ratio is given by $\{(1 + \beta_e \cos \phi)/(1 - \beta_e \cos \phi)\}^{2+\alpha}$, where $v_e = \beta_e c$ is the bulk velocity of the extended emission, ϕ is the angle of inclination of the source axis to the line of sight and α is the radio spectral index, defined by the relationship between flux S and frequency ν : $S \propto \nu^{-\alpha}$. Examples of core-dominated, one-sided sources are NRAO140 (0333+321) (refs 17, 18), 3C273 (1226+023) (ref. 19) and 3C454.3 (2251+158) (refs 4, 17), which are also well-known superluminal sources⁸.

In order to make a systematic study of one-sided radio sources, we have been making maps with high angular resolution and good dynamic range of all known candidates with both MER-

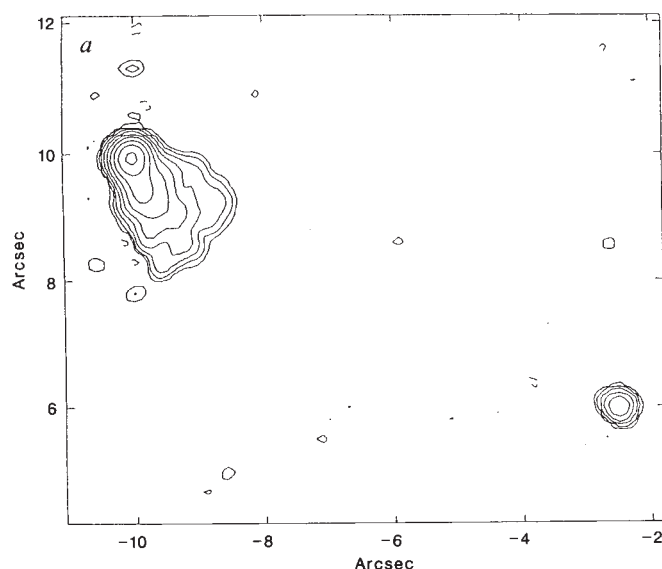
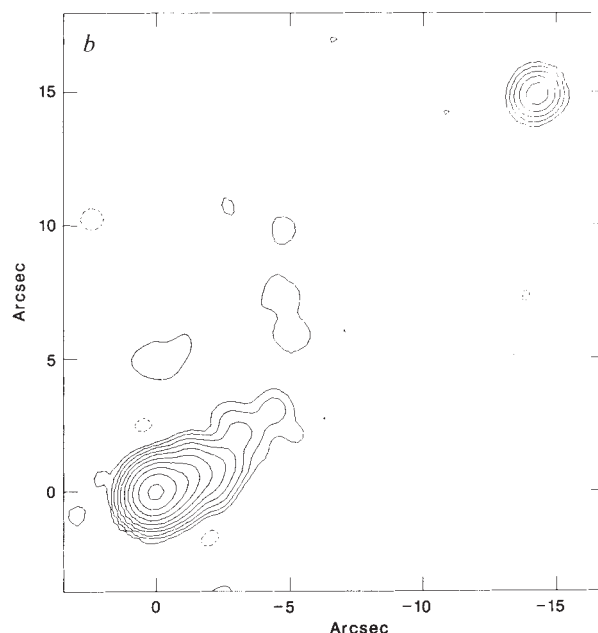


FIG. 1 a, MERLIN map of 0404+177 at a wavelength of 18 cm. Peak brightness=121 mJy per beam. Contours are $0.4 \times (-1, 1, 2, 4, 8, 16, 32, 64, 128, 256)$ mJy per beam. Restoring beam = 0.3×0.3 arcsec. b, MERLIN



map of 1729+501 at a wavelength of 73 cm. Peak brightness=1,236 mJy per beam. Contours are $2 \times (-1, 1, 2, 4, 8, 16, 32, 64, 128, 256, 512)$ mJy per beam. Restoring beam = 1×1 arcsec.

TABLE 1 Properties of the one-sided sources

Source	Alternative name	Redshift	Peak flux/ 3σ	f_c	Largest angular size (arcsec)	Projected linear size (kpc)
0404+177	4C17.22	1.712	435	0.02	8.6	72
1559+173	4C17.65	1.944	690	0.05	2.8	23
1629+120	4C12.59	1.795	665	0.13	2.0	17
1729+501	4C50.43	1.107	810	0.07	20.7	178
0333+321	4C32.14	1.258	20	0.99	7.6	65
1226+023	3C273	0.158	3,800	0.89	21.7	77
2251+158	3C454.3	0.859	180	0.96	5.4	45

LIN and the Very Large Array (VLA). Our observations have shown that many of the candidates were misclassified as one-sided because of poor surface-brightness sensitivity or low angular resolution of the earlier observations. From our observations we have also been able to identify a small class of one-sided sources whose lower limits on the degree of asymmetry are amongst the highest known, but which are dominated by their extended emission. We have observed the four known members of this class, namely 0404+177, 1559+173, 1629+120 and 1729+501, with MERLIN and the VLA. The first three were observed using MERLIN, with an 18-m telescope at Cambridge comprising part of the array. The higher angular resolution obtained when using the Cambridge telescope proved to be essential in distinguishing the lobe emission from that of the core in the small-angular-sized source 1629+120.

To illustrate their radio structures, MERLIN maps of two of the sources, namely 0404+177 and 1729+501, are shown in Fig. 1a and b respectively. VLA snap-shot images of these two sources have been published earlier^{20,21}. Observed properties for the four lobe-dominated one-sided sources, as well as for the three core-dominated ones mentioned earlier, are summarized in Table 1. We list, as a quantitative estimate of the degree of asymmetry of the source, the ratio of peak brightness of the detected lobe to three times the r.m.s. noise on the opposite side. We also list the fraction of emission from the core f_c , at a frequency of 8 GHz (which can be used as a statistical measure of source orientation in the relativistic beaming models), the largest angular size of the source in arcsec, and its projected linear size, l , in an Einstein-de Sitter universe with Hubble constant $H_0 = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$.

The lobe-dominated, one-sided sources are all identified with quasars whose optical positions are coincident with those of the radio cores. It is extremely unlikely that these cores, with spectral

indices of ≤ 0.5 between frequencies of 408/1,665 and 5,000 MHz, are unrelated to the lobes. Using the fraction of flat-spectrum quasars at different flux-density levels given by Orr and Browne¹², we estimate that the number expected by chance is less than 0.01 in about 1,000 sources. In addition, the morphology and orientation of the lobes also suggest that they are physically related.

To illustrate the striking difference between these lobe-dominated, one-sided sources and all other one- and two-sided quasars, we plot in Fig. 2 the degree of asymmetry against f_c for 3C/4C quasars that have both a measured redshift and a good-quality radio image (as determined either in this study, or by Bridle *et al.* (personal communication), or in data presented in refs 17 and 22–24). For two-sided quasars we have plotted the peak brightness ratio of the two lobes if available, or the flux-density ratio otherwise. The brightness ratios are obtained from observations with angular resolutions better than ~ 1 arcsec. The most asymmetric source is 3C273, for which we suggest that the lobe south of the jet may be the counter-lobe seen in projection. If so, it should depolarize more rapidly than the approaching jet, in accordance with the correlation of lobe depolarization with jet sidedness^{25,26}. Observations to determine the polarization characteristics of this feature are required to investigate this possibility.

Most of the one-sided sources in Fig. 2 are clustered in the high- f_c region (median $f_c \approx 0.9$) with a median degree of asymmetry of ~ 40 , which implies, in the simple relativistic beaming models, a hot-spot speed of $0.6c$ for a source inclined at $\sim 15^\circ$ to the line of sight. The opposite corner of the figure is populated by the weak-cored, one-sided sources with $f_c \leq 0.15$ and a lower limit to the degree of asymmetry ranging from ~ 450 to 800.

It is important to determine whether these weak-cored, one-sided sources are simply intrinsically one-sided, whether they

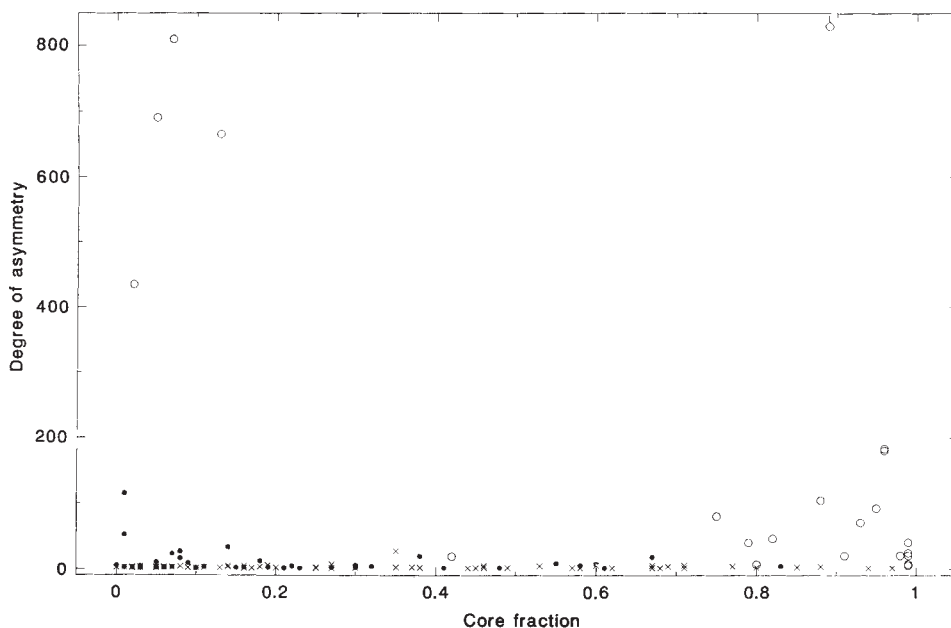


FIG. 2 The degree of asymmetry plotted against the fraction of emission from the core, f_c , at an emitted frequency of 8 GHz for 3C/4C quasars. For the one-sided sources ($\circ$), these values are the ratio of the peak brightness to three times the r.m.s. noise on the opposite side, and for the two-sided quasars they are either the peak brightness ratio for the lobes if the values are available ($\bullet$), and the lobe flux-density ratio ($\times$) otherwise. The point on the upper right-hand corner is 3C273, whose degree of asymmetry is $\sim 3,800$.

are examples of sources in which energy is supplied alternately on opposite sides but with a low 'flip-flop' rate²⁷, or whether they can still be understood in relativistic beaming models by modifying the basic unified scheme. When the unified scheme was developed, it seemed reasonable to assume that the extended emission is essentially at rest relative to the nuclear emission, which could be moving close to the velocity of light¹⁰⁻¹³. However, if the observed one-sidedness of the lobe-dominated sources is due to relativistic beaming, then the velocities required for the extended emission are at least about $0.8c$ even for a source inclined at $\sim 15^\circ$ to the line of sight. For reasonable values^{12,13} of the bulk Lorentz factors of the nuclear jets ($\gamma_c \approx 5$) and $F_c \equiv f_c(\phi = 90^\circ) = 0.033$, the expected value of f_c using the expression given by Kapahi and Saikia¹³ is 0.86 for $\phi = 15^\circ$ if $\beta_e = 0$. This is clearly not consistent with the values observed for the lobe-dominated, one-sided sources. Modifying the basic unified scheme to include relativistic beaming of the extended emission changes the expression for the expected value of f_c to $1/[1 + \{B_e(\phi)/B_c(\phi)\} \{(1/F_c) - 1\}]$, where $B = (1 - \beta \cos \phi)^{-(2+\alpha)} + (1 + \beta \cos \phi)^{-(2+\alpha)}$, with subscripts e and c denoting extended and core (or nuclear) radio emission. Assuming the spectral indices of the core and extended radio emission to be 0 and 1 respectively, the value of f_c decreases from 0.86 when $\beta_e = 0$ to 0.12 when $\beta_e = 0.8$ for the same set of parameters mentioned earlier. Here, the beamed extended emission dominates the beamed core emission, which is intrinsically weaker. It is thus possible to understand the brightness asymmetry of the lobes in the lobe-dominated, one-sided sources, and also their low values of f_c in the unified scheme, if we are prepared to widen it to include very significant beaming of the extended emission for a small fraction of sources.

However, besides necessitating such changes in the scheme, there are other problems with this explanation. Even if the hot-spots are moving with such high velocities, it is difficult to understand the asymmetry of the well developed lobes (Fig. 1), which should comprise mainly backflow from the hot-spots. Also, if there is a range of hot-spot speeds extending up to $\sim 0.8c$ for a small fraction of sources, it is not clear why there are no intermediate cases with, say, $f_c \approx 0.5$ and degrees of asymmetry of several hundred. Further observations, including those aimed at detecting very weak diffuse emission on the opposite side of the one-sided sources, are clearly required to help clarify the situation.

An important diagnostic to distinguish between different explanations for these lobe-dominated, one-sided sources is likely to be provided by VLBI observations of their nuclei. If they are indeed inclined at small angles to the line of sight, rather than being simply intrinsically one-sided sources observed at large viewing angles, one would expect to find evidence of phenomena more characteristic of core-dominated radio sources. These include significant misalignments between the VLBI structures and the arcsecond-scale structures, which are well aligned in lobe-dominated sources^{1,6,7}, and evidence of superluminal motion⁸. One would also expect to observe significant variability of the core flux density, lack of any correlation of core polarization vector with the radio-source axis²⁸ and low equivalent width of the emission lines²⁹. On the other hand, if the VLBI jets are on the opposite side to that of the arcsecond-scale structure, these sources would provide perhaps the best examples of the 'flip-flop' model. In this model, energy is supplied alternately on opposite sides but, averaged over its lifetime, the source appears reasonably symmetric. The timescale for the energy to change direction would have to be at least $\sim 10^5$ years for the lobe-dominated, one-sided sources. $\square$

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Evidence for a molecule heavier than methane in the atmosphere of Pluto

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THE recent occultation of a 12th magnitude star by Pluto provided a unique opportunity for studying its atmosphere. Analyses of measurements made at the Hobart observatory in Australia and the Kuiper Airborne Observatory (KAO) have been published^{1,2}. It is generally agreed that Pluto possesses a substantial atmosphere, with a surface pressure of $\sim 10 \mu\text{bar}$. Both occultation measurements are sensitive primarily to the atmospheric conditions at the $1\text{-}\mu\text{bar}$ level. Analysis of the Hobart data reveals an atmospheric scale height of 46-57 km at a radial distance of 1,240-1,290 km, whereas the scale height derived from the KAO data is 59.7 ± 1.5 km at $1,214 \pm 20$ km. The existence of an optically thick dust layer along the line of sight at the limb has been inferred from the KAO measurements, raising doubts about the true surface radius of Pluto². The measured scale heights are consistent with a purely methane atmosphere at a temperature of 50-61 K for the Hobart data¹ and 67 ± 6 K for the KAO data². These values are close to the surface temperature³. Here we examine the energy balance in the atmosphere and conclude that the temperature near $1 \mu\text{bar}$ is ~ 100 K rather than the surface temperature; consequently, the mean molecular weight of the atmosphere is close to 25 a.m.u., and a molecule heavier than (and in addition to) methane must be present in the atmosphere.

Methane, or methane ice, has been detected spectroscopically in the Pluto atmosphere but it is difficult to determine the atmospheric abundance from these data⁴. Upper limits of $\sim 25 \mu\text{bar}$ have been placed on the gaseous abundance of CH_4 from the infrared spectrum^{3,5}. However, even small amounts of CH_4 are sufficient to cause substantial heating of an atmosphere. The CH_4 molecule possesses a number of strong vibrational bands in the thermal infrared and is believed to be a major source of stratospheric heating on the outer planets and Titan⁶⁻⁸.

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Therefore, it seems likely that methane will also be an important heating agent for Pluto's atmosphere and that the atmospheric temperature may be elevated above the surface temperature. We study the possibility of significant atmospheric heating due to the presence of CH_4 by constructing thermal models for Pluto's atmosphere, including infrared heating and cooling through the CH_4 bands and thermal conduction to the surface.

The strongest CH_4 bands occur at 3.3 and 7.8 μm . The 7.8- μm band, which connects the ground state to the fourth vibrational (ν_4) level, is the lowest-lying fundamental and, at the low temperatures on Pluto, will be the dominant channel for radiant energy loss. Examination of the solar spectrum and band strengths reveals that the bulk of the heating will occur in the 3.3- μm band, which connects the ν_3 level to the ground state, although the bands at 1.7 and 2.3 μm may make an important contribution^{7,8}. Because of the low temperatures and low collision rates, the atmosphere will be far from thermodynamic equilibrium and we must solve the energy-transport equation to determine the atmospheric temperature profile. The steady-state energy transport equation, ignoring convective terms, is

$$-\frac{d}{dz} \kappa \frac{dT}{dz} = Q - L \quad (1)$$

where T is the temperature, κ is the thermal conductivity, z is the altitude, Q is the heating rate, and L is the radiative cooling rate. Equation (1) ignores effects such as the variation of gravity with altitude and adiabatic cooling, which are important in the thermosphere, because our intention is to estimate the temperature in the bottom layers of the atmosphere. The validity of these assumptions is discussed later.

The radiative cooling rate in an optically thin atmosphere may be expressed as

$$L = g n h \nu A_R \exp\left(-\frac{h\nu}{kT}\right) \quad (2)$$

where n is the number density of the radiating constituent, $h\nu$ is the energy of the emitted photon, k is Boltzmann's constant, g is a factor that accounts for the statistical weights of the upper and lower states, and ϵ represents the efficiency at which vibrational energy is converted into radiation and is determined by the relative values of the radiative decay rate and vibration-translation (V-T) reaction rate,

$$\epsilon = \frac{P_{10} Z n_a}{P_{10} Z n_a + A_R} \quad (3)$$

where n_a is the atmospheric density, Z is the collision rate, P_{10} is the probability that a V-T transition occurs during a collision, and A_R is the Einstein coefficient for the band in question. V-T probabilities for CH_4 - CH_4 , CH_4 - CO , and CH_4 - N_2 collisions at room temperature are 5.3×10^{-5} , 9.4×10^{-6} and 5.5×10^{-6} respectively⁹. At the low temperatures on Pluto the V-T probability is likely to be smaller, but experimental data are lacking and extrapolations are uncertain. To make the extrapolation we follow the procedure outlined by Lellouch *et al.*¹⁰, which results in a decrease by a factor of ~ 10 in P_{10} in extrapolating from 300 K to 100 K. The factor of 10 is adopted as an indication of the uncertainty in P_{10} , and we examine the dependence of the thermal-structure calculations on this quantity. We note that pressure-induced transitions are far too weak to cause significant cooling at pressures of $\sim 1 \mu\text{bar}$.

The radiative heating rate is given by

$$Q = \frac{\pi F}{4} \epsilon n B \quad (4)$$

where πF is the solar flux at 3.3 μm , B is the band strength, and the factor of $\frac{1}{4}$ accounts for global averaging. In equation (4), ϵ is given by an expression similar to equation (3), although the probability for deactivation and the radiative decay rate may

be different because heating and cooling occur through different bands. The assumption of optical thinness for the heating and cooling rates may be justified in the manner described by Dickinson¹¹.

Aerosol heating is thought to be an important factor in the stratospheric thermal balance on Titan and the outer planets, and may also play a role on Pluto⁷⁻⁹. If aerosol heating is important it could mean that the temperatures are significantly higher than those calculated here. At present there is insufficient information to quantitatively evaluate this possibility.

Using equations (2) and (4) for the heating and cooling rates, we solve equation (1) for the temperature profile. We assume that the atmosphere is composed of CH_4 and a species of atomic weight 28 in diffusive equilibrium. (We use a 3.3- μm band strength of $0.30 \mu\text{m cm-atm}^{-1}$ and a solar flux of $23 \text{ erg cm}^{-2} \text{ s}^{-1} \mu\text{m}^{-1}$. The radiative decay rate of the 7.8- μm band is 2.56 s^{-1} whereas a radiative decay rate of 4.24 s^{-1} is adopted for the group of bands centred at 3.3 μm .) We adopt the 10- μbar level as the lower boundary and the $\sim 10^{-2}$ - μbar level as the upper boundary. The heat flux at the upper boundary is set to zero despite the fact that most of the solar extreme-ultraviolet energy will be deposited above this level. This is because a large fraction of the energy deposited in the thermosphere may be lost through hydrodynamic escape of the atmosphere¹². Solution of the hydrodynamic equations is necessary to determine what fraction of the solar extreme-ultraviolet energy flows downward into the lower atmosphere, but such a calculation is beyond the scope of this paper. Calculations with a downward heat flux at the upper boundary show that conditions near 1 μbar are insensitive to thermospheric heat flows of $5 \times 10^{-4} \text{ erg cm}^{-2} \text{ s}^{-1}$. The temperature and pressure at the surface of Pluto are uncertain and the values given above are simply reasonable estimates. Fortunately, the calculations demonstrate that the conditions at the lower boundary have a very small effect on the conditions at 1 μbar , provided that the surface pressure is greater than $\sim 3 \mu\text{bar}$.

Results of the thermal-structure calculations are summarized in Table 1 and an example is shown in detail in Fig. 1. We note that the temperature profile is close to isothermal in the 1- μbar region, as assumed in the occultation analysis. In general the temperature at 1 μbar is insensitive to both P_{10} and the CH_4 mole fraction. P_{10} has little effect because the heating and cooling rates depend on this quantity in the same way, and consequently lower heating efficiencies are accompanied by lower cooling

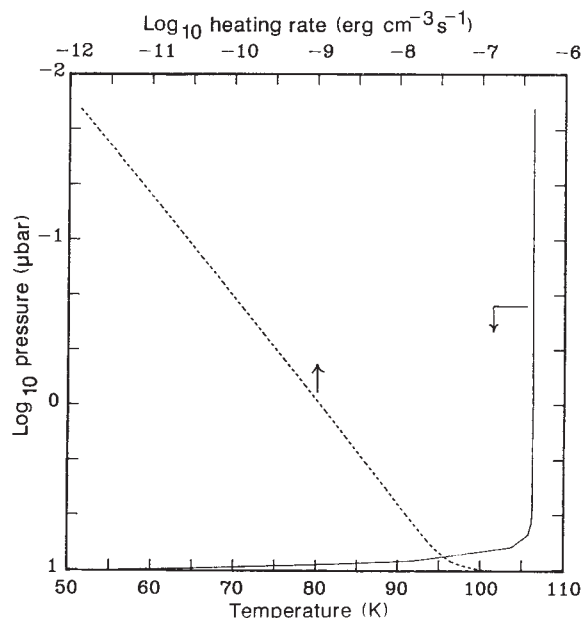


FIG. 1 The temperature profile and infrared heating rates for a model atmosphere with $P_{10} = 10^{-6}$ and a CH_4 mole fraction of 10%.

efficiencies. Similarly, both heating and cooling rates are directly proportional to the methane mole fraction and its effects tend to cancel. These arguments break down when the radiative time constants become so small that the conduction term in equation (1) becomes important and heat flows to the surface faster than it is radiated away. In our models this occurs only for the smallest combinations of P_{10} and CH_4 mole fraction.

To assess the dependence of thermal structure on the infrared heating rates, we performed calculations with heating rates reduced by a factor of 3. The temperature at 1 μbar decreased by 6% for the model with a CH_4 mole fraction of 10% and $P_{10} = 10^{-6}$; consequently, the approximations used to drive these rates should not cause much concern. We have also modelled the effects of adiabatic cooling on the thermal structure. We find that an escape flux of less than $1 \times 10^{12} \text{ cm}^{-2} \text{ s}^{-1}$, referred to the surface, produces no noticeable effects near 1 μbar . This flux is much larger than previously estimated upper limits to the escape rate¹².

We can use the measured scale height with the thermal-structure calculations to infer the mean mass of the Pluto atmosphere near 1 μbar . A temperature of 106 K and scale height of 59.7 ± 1.5 implies a mean mass of 25 ± 3 , which is much heavier than CH_4 and implies that the major atmospheric constituent is a heavier gas such as CO , N_2 or argon. If the heavier gas is argon, the error bars on the scale height imply a CH_4 mole fraction between 40 and 65%. If the heavier gas has a molecular weight of 28 the upper bound on the CH_4 mole fraction is 42%. The lower bound is determined by the amount of CH_4 required to produce a temperature on the order of 100 K. From the results in Table 1 we estimate a very conservative lower limit of 0.1%.

To estimate the relative abundance of CH_4 and the heavier gas in the atmosphere and surface requires modelling the relative escape fluxes and photolytic/cosmic-ray destruction rates of these gases; this requires more-detailed knowledge than we have at present. Likewise, it is not clear whether the lower atmosphere of Pluto is well mixed, whether both constituents are in equilibrium with surface ices, or whether diffusive separation of the gaseous components has significantly enhanced CH_4 at the occultation level. The range of methane compositions derived above (0.001–1), however, is sufficiently large that these considerations are not critical for the discussion of cosmochemistry that follows.

Based on solar elemental abundance, the most likely candidates for the heavy-gas component of Pluto's atmosphere are argon, carbon monoxide and molecular nitrogen. Models of the incorporation of argon and nitrogen into outer-Solar-System bodies by direct condensation or clathration predict that the argon-to-nitrogen ratio should be ≤ 0.1 , the solar elemental ratio; we have no evidence to the contrary (for example, from Titan⁴) and hence we assume that argon is a secondary component. The presence of a predominantly nitrogen-methane atmosphere on Titan would suggest that nitrogen is a good candidate for the heavy gas. However, the uncompressed density of Titan ($1.5\text{--}1.6 \text{ g cm}^{-3}$) is low relative to that of Pluto ($>1.99 \text{ g cm}^{-3}$); also Titan's position as a regular satellite of Saturn suggests a different origin for Titan than for Pluto^{4,13}. In particular, Titan formed in a water-rich nebula, and incorporated methane and ammonia, which over the age of the Solar System was converted to molecular nitrogen by photolysis and shock heating of its atmosphere⁴. Pluto's high density and position as a solar-orbiting body suggest, however, that it was formed

in the relatively water-poor solar nebula¹⁵. Models predict that carbon monoxide was the dominant carbon-bearing molecule in the nebula. If so, then the heavy gas in Pluto's atmosphere could be carbon monoxide. The question may then be posed: is the inferred ratio of methane relative to carbon monoxide, q_P , consistent with an origin in the solar nebula?

The most plausible mechanism for bringing these gases into Pluto during its formation is trapping in water ice by adsorption or clathration, as nebular models do not predict temperatures low enough to condense the pure solids of these species¹³. Let q_N be the ratio in the solar nebula of CH_4 to CO . The values of q_N and q_P are not identical because the gases are trapped to differing extents in water ice, determined by the number of available adsorption sites and the polarizability of the two gas species. A conservative fractionation factor q_N/q_P is 0.1–0.01. Hence, based on the atmospheric abundance ratio derived above (0.001–1), q_N could range from 10^{-1} to 10^{-5} in the region of the solar nebula from which Pluto formed. These values are consistent with chemical models for the solar nebula^{16,17}. Moreover, as the water abundance is tied directly to the carbon monoxide abundance in such nebular models, the density of Pluto can be calculated for this range of q_N values¹⁴. One finds a Pluto density of $1.99\text{--}2.08 \text{ g cm}^{-3}$, consistent with the lower bound on the density derived from Pluto-Charon eclipse data³ (which is only a lower bound because of uncertainties in the surface radius and the relative densities of Pluto and Charon¹⁸). It is interesting that q_P overlaps with the methane/carbon monoxide ratio (~ 0.3) found in comet Halley¹⁷. From those data, it has been concluded that the final stages of formation of Halley also took place in the solar nebula¹⁷. Although we cannot assert that the atmospheric ratio is reflective of the bulk ratio in Pluto, the arguments presented above illustrate the expectation that Pluto should have a substantial amount of CO .

The assumption that the heavier gas in Pluto's atmosphere is largely CO is consistent with cosmochemical models of the solar nebula and Pluto's density. If our interpretation is correct, nebular chemical models would predict the presence of molecular nitrogen in Pluto's atmosphere, at an abundance roughly 10–100 times less than that of carbon monoxide and an argon abundance of $\sim \frac{1}{10}$ of that of nitrogen. Although we cannot rule out a purely methane-nitrogen atmosphere, like that of Titan, the bulk density of Pluto, its position as a solar-orbiting object, and the results of the comet Halley measurements do not support this.

Much of this discussion of Pluto's thermal structure can be applied directly to Triton, which may be approximately the same size, and also shows evidence for surface, and hence atmospheric, methane. Both the composition and temperature of Triton's atmosphere will be measured during the Voyager encounter, providing a potential test of the ideas presented here. $\square$

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TABLE 1 Temperatures (K) at 1 μbar

P_{10} \ n/n_a	100%	10%	1%	0.1%
10^{-5}	106	106	106	101
10^{-6}	106	106	102	63
10^{-7}	106	102	63	56

Superconductivity at 3.5 K in $\text{BaPb}_{0.75}\text{Sb}_{0.25}\text{O}_3$: why is T_c so low?

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SUPERCONDUCTING $\text{BaPb}_{0.75}\text{Bi}_{0.25}\text{O}_3$, with a transition temperature (T_c) of 12 K (ref. 1), is now widely viewed as the precursor to the recent appearance of copper oxide superconductors with record high T_c s. The observation of superconductivity near 30 K in $\text{Ba}_{0.6}\text{K}_{0.4}\text{BiO}_3$ (refs 2,3) has raised serious questions as to whether conventional electron-phonon coupling is the primary pairing mechanism in the bismuth-lead oxide perovskites. Their T_c s are anomalously high, given their low densities of electronic states at the Fermi level^{4,5}, but the debate as to the mechanism of superconductivity is far from settled (see, for example, refs 6–8). Here we report the occurrence of superconductivity at 3.5 K in a new member of the family, $\text{BaPb}_{0.75}\text{Sb}_{0.25}\text{O}_3$. Its T_c , less than one-third that of $\text{BaPb}_{0.75}\text{Bi}_{0.25}\text{O}_3$, is surprisingly low in view of the similarities between the two compounds. The discovery of superconductivity in $\text{BaPb}_{0.75}\text{Sb}_{0.25}\text{O}_3$ establishes a series of isostructural compounds with T_c s differing by an order of magnitude. This series provides the basis for further studies to elucidate the essential electronic characteristics that lead to high T_c in the bismuth oxide compounds.

The $\text{BaPb}_{1-x}\text{Sb}_x\text{O}_3$ perovskites were synthesized in polycrystalline form to determine the optimal superconducting stoichiometry. Starting materials BaCO_3 , Pb_3O_4 and Sb_2O_3 were mixed and ground mechanically for 30 min in stoichiometric

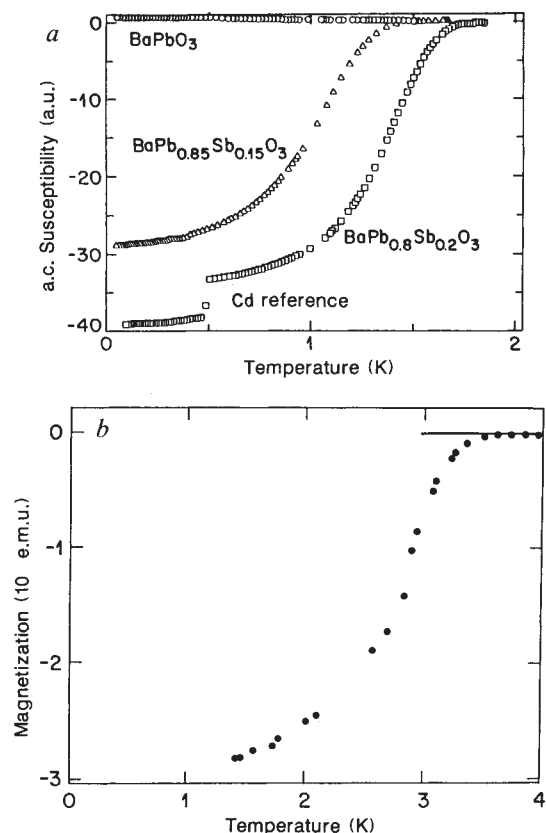


FIG. 1a, Real part of the a.c. susceptibility at 1 kHz, showing the evolution of the superconducting transition in polycrystalline $\text{BaPb}_{1-x}\text{Sb}_x\text{O}_3$ at low Sb concentrations. a.u. denotes arbitrary units. b, The superconducting transition for small single crystals of $\text{BaPb}_{0.75}\text{Sb}_{0.25}\text{O}_3$ measured in a d.c. magnetometer in a field of 5 Oe.

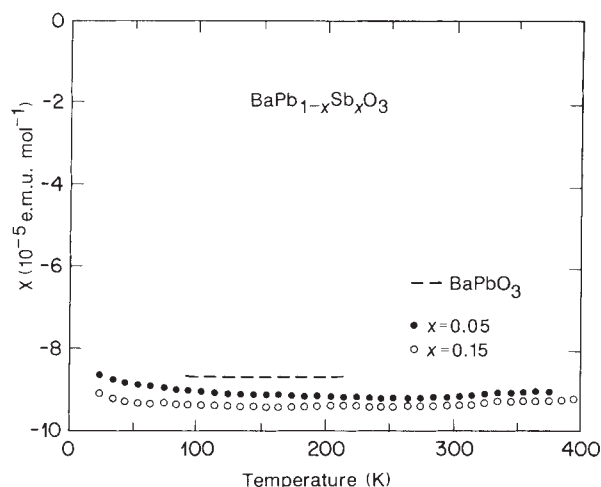


FIG. 2 Normal-state magnetic susceptibilities (χ) for $\text{BaPb}_{1-x}\text{Sb}_x\text{O}_3$ at $x = 0.05$ and $x = 0.15$, measured at 30 kOe, compared with that of BaPbO_3 .

ratios for $0 \leq x \leq 0.50$. Reactions were carried out in dense Al_2O_3 crucibles under flowing O_2 . Initial reactions were in powder form at 825 °C for 12 h. The powders were then ground mechanically for 30 min and re-reacted in O_2 for 5 h at the same temperature. After another grinding, a portion of each powder was then pressed into a ceramic pill, buried in the powder of its own composition, and fired in the Al_2O_3 crucibles again for 5 h at 825–850 °C in O_2 . These reaction steps are performed at low temperatures because the solubility limit of Sb into BaPbO_3 appears to decrease significantly at higher temperatures (and lower P_{O_2}). The burying procedure guards against excessive volatilization of Pb. The resultant pills are not dense, and are extremely fine-grained. The samples cool in the O_2 flow from 850 to 200 °C in ~ 1 h. Long-time, low-temperature (400 °C) O_2 anneals degrade the superconducting properties of the samples.

Superconductivity with the highest T_c occurs in $\text{BaPb}_{1-x}\text{Bi}_x\text{O}_3$ for the composition $x = 0.25$, close to the metal-semiconductor transition at $x = 0.35$ (refs 9,10). Both endmembers, BaPbO_3 and BaBiO_3 , have perovskite structures. The same does not hold for the antimony system, for which the stable barium antimony oxide is BaSb_2O_6 . Therefore a complete perovskite-type solid solution is not expected. For the samples prepared as described, the $\text{BaPb}_{1-x}\text{Sb}_x\text{O}_3$ phase exists for all $x \leq 0.50$. Although X-ray diffraction of some samples showed very weak impurity peaks ($< 3\% I_{\text{max}}$), the fact that they did not increase with Sb content, and the fact that the crystallographic cell changed systematically over the whole range of composition, lead us to conclude that the perovskite exists to the composition $\text{Ba}_2\text{PbSbO}_6$.

Single crystals with $x = 0.25$ (as measured by inductively coupled plasma analysis) were grown from a PbO-based flux. These were blue-black in colour, and roughly equi-dimensional (1–2 mm), with some having an octahedral morphology. The starting composition was: 48.080 g PbO, 13.515 g BaCO_3 , 1.456 g Sb_2O_3 . The Sb has a high segregation coefficient into the crystals, and therefore local inhomogeneities in the melt may lead to significant changes in the Sb content of the crystals. Bracketing the optimal charge composition by $\pm 15\%$ Sb in parallel growth runs was therefore found to be preferable. The starting composition was thoroughly mixed, placed in a covered platinum crucible and heated to, 1,150 °C. After a 3-h soak time, the melt was cooled to 750 °C at 4 °C h^{-1} , held at 750 °C for 24 h and then removed from the furnace. The crystals were separated from the solidified flux mechanically.

In Fig. 1a we show the evolution of the superconducting transition at low Sb contents. The magnetic transitions were measured on pressed powder compacts at 1 kHz with an a.c. susceptibility technique, in a dilution refrigerator. From com-

parison with a Cd standard of known volume, we infer that superconductivity in Ba(Pb, Sb)O₃ is a bulk effect. We have not observed a superconducting transition in BaPbO₃ down to temperatures as low as 30 mK, even though superconductivity below 0.5 K has previously been reported¹¹. For BaPb_{1-x}Sb_xO₃ in polycrystalline form, T_c increases smoothly with Sb content, reaching a maximum onset temperature of ~3 K at compositions near $x = 0.30$. For the single crystals of composition $x = 0.25$, the transition is high enough in temperature to be fully observed in a commercial d.c. magnetometer (SHE 905). Single crystals with $x \geq 0.35$ do not show superconducting transitions above 1.5 K. Figure 1b shows the magnetization from a collection of single crystals ($x = 0.25$) cooled in a field of 5 Oe. T_c is 3.5 K. Even in these low fields, pronounced time-dependence in the magnetization $M(T)$ is observed, indicative of flux creep. For example, after cooling from 2 K to 1.5 K, the excluded flux increases by 100% within half an hour. After proper consideration of demagnetization effects, the observed signal corresponds to 20% of the ideal Meissner effect. The d.c. magnetization signal is strongly reduced in the fine-grained, compressed powder samples, because the grain size is comparable to the magnetic penetration length.

Figure 2 shows as examples the normal-state magnetic susceptibility $\chi(T)$ for polycrystalline samples of BaPb_{1-x}Sb_xO₃ at $x = 0.05$ and 0.15, measured in a field of 30 kOe. Data for BaPbO₃ are also shown for comparison. As in the related compounds Ba(Pb, Bi)O₃ and (Ba, K)BiO₃, the susceptibility is negative, only very slightly temperature-dependent, and dominated by core diamagnetism. When carriers are introduced into BaPbO₃ by Sb substitution, χ decreases slightly, as occurs in Ba(Pb, Bi)O₃ (ref 12). The visual appearance of the materials reflects changes in the electronic properties: powdered BaPbO₃ is dark brown in colour; with the substitution of as little as 0.05 Sb, the colour changes to blue-black; this colour deepens continuously as the Sb content increases; and for Sb contents greater than $x = 0.4$ the colour turns black.

The symmetry of the crystallographic unit cell changes with Sb content. The ideal undistorted ABO₃ perovskite structure consists of a three-dimensional array of regular BO₆ octahedra

with 180° B-O-B angles. The A atom is at the centre of the resultant 12-coordinate cavity. The ideal perovskite has simple cubic symmetry, but rotations of the octahedra, leading to larger unit cells and lower symmetry, are common. In BaBiO₃, however, larger and smaller BiO₆ octahedra alternate¹³, driven by the tendency of the charge in Bi-O bonds to disproportionate and to form a charge density wave. For BaPbO₃, the symmetry is orthorhombic ($a = 6.024$, $b = 6.065$, $c = 8.506$ Å), and involves primarily conventional rotational distortions^{14,15}. In the BaPb_{1-x}Sb_xO₃ solid solution, the magnitude of this orthorhombic distortion decreases with increasing Sb concentration, through the decrease of the b -axis length. The X-ray powder diffraction peaks most characteristic of this change are compared in Fig. 4 for BaPbO₃ and a powdered single crystal of BaPb_{0.75}Sb_{0.25}O₃. The 404 and 044 reflections of BaPbO₃ are resolved because $a \neq b$. In BaPb_{0.75}Sb_{0.25}O₃ they have collapsed into a single peak, indicating that $a = b$. The 440 and 008 reflections of both BaPbO₃ and BaPb_{0.75}Sb_{0.25}O₃ are resolved because c is not a simple multiple of the 110 dimension. The symmetry of BaPb_{0.75}Sb_{0.25}O₃ is therefore tetragonal, with $a = b = 6.028$ Å and $c = 8.511$ Å, estimated from the positions of the peaks in Fig. 3. For polycrystalline materials in the range $0.35 \leq x \leq 0.45$, the 440 and 008 peaks coalesce, suggesting cubic symmetry with a cell parameter $a = 4.254$ Å at $x = 0.40$. At higher Sb contents, the symmetry becomes lower, with a pseudo-cubic cell parameter $a = 4.248$ Å at $x = 0.50$.

In Fig. 4 we compare our experimental estimates for the pseudo-cubic crystallographic cell parameters, a_0 , for BaPb_{1-x}Sb_xO₃ with those observed for BaPb_{1-x}Bi_xO₃ (ref 16). Also shown are the calculated pseudo-cubic parameters expected for solid solutions of Bi and Sb in BaPbO₃ according to ref 17. The observed variation of a_0 with Bi content in Ba(Pb, Bi)O₃ follows very closely what is expected if Bi is accommodated in a mixed-valent state comprising an equal mixture of formal Bi(III) and Bi(V), as required by charge neutrality. The same type of behaviour is also observed for the Ba(Pb, Sb)O₃ solid solution, suggesting that the Sb also may be accommodated in a mixed-valent state. Microscopic probes of the Pb-O and Sb-O bonds are necessary to estimate the formal charge states of the electronically active ions.

The T_c of 3.5 K in BaPb_{0.75}Sb_{0.25}O₃ is particularly remarkable when compared to the T_c of 12 K in BaPb_{0.75}Bi_{0.25}O₃ and of 30 K in Ba_{0.6}K_{0.4}BiO₃. The Sb-O bond might generally be considered to be more ionic than the Bi-O bond in oxides. In the present compound, however, the degree of covalency of the Sb-O bond might be similar to that of the Bi-O bond in BaBiO₃. This can be inferred from the relative atomic energy levels of Pb, Bi and Sb, with the Sb 5s level being only 0.8 eV lower than the Bi 6s, which in turn is 2 eV lower than that of Pb (ref. 18).

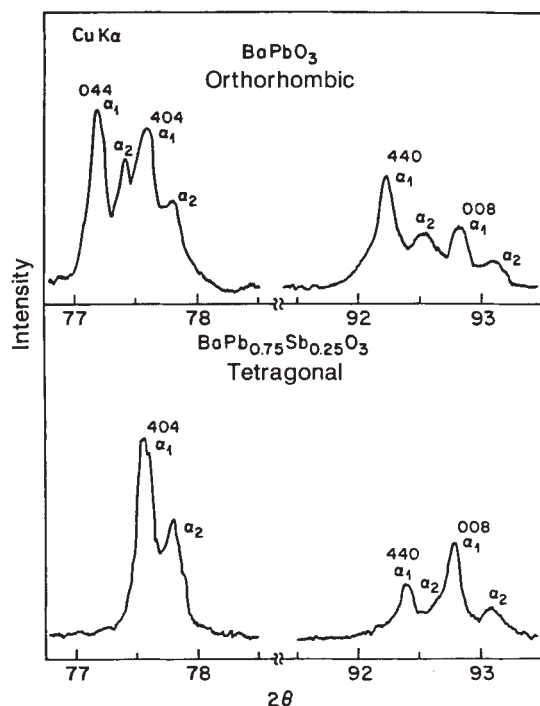


FIG. 3 Comparison of characteristic regions of the powder X-ray diffraction patterns (Cu K α) of BaPbO₃ and pulverized superconducting BaPb_{0.75}Sb_{0.25}O₃ single crystals.

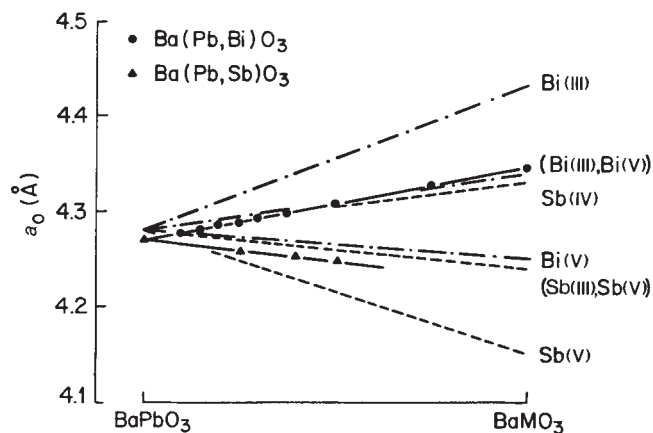


FIG. 4 Observed and calculated pseudo-cubic lattice parameters in Ba(Pb,M)O₃. Symbols and solid lines represent experimental data, and dashed lines are calculated for the solid solutions of the indicated ion or mixtures of ions in BaPbO₃, according to the method of ref. 17.

These differences are small relative to the width of the conduction band derived from the metal- s -oxygen- p σ -bonds¹⁹. Furthermore, the variation of lattice parameters in the Ba(Pb, Sb)O₃ solid solution obeys the same principle as that observed in Ba(Pb, Bi)O₃. In both cases, the implied average Bi-O or Sb-O bond length is that expected if these atoms are accommodated into BaPbO₃ in a mixed-valent state. More detailed study is necessary to determine whether that is indeed the case in the current material. The same tendencies for bond charge disproportionation⁸ may therefore be acting in both Ba(Pb, Sb)O₃ and Ba(Pb, Bi)O₃. Given that their crystal structures, phononic properties, chemical bonding and formal electron counts are so similar, it is surprising that their superconducting T_c s differ by more than a factor of three. Additional microscopic studies will be necessary to determine whether special characteristics of Sb-O bonding are essential for superconductivity in Ba(Pb, Sb)O₃ or whether Sb is simply acting as an inert electron donor. The fact that we have been unable to synthesize the analogous Ba(Pb, Nb)O₃ compound suggests that the latter is not the case. Further comparison with (Ba, K)BiO₃ may help to establish why T_c in that compound is so unusually high. □

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Anomalous absence of pressure effect on transition temperature in the electron-doped superconductor Nd_{1.85}Ce_{0.15}CuO_{4-δ}

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THE recent discovery of the electron-doped superconductors¹ added fresh fuel to controversies about the mechanism of high-temperature superconductivity in copper oxide compounds. The superconducting transition temperature (T_c) of almost all the hole-doped copper oxide compounds increases with increasing

pressure². The pressure coefficient has a large positive value compared with a typical conventional (BCS) superconductor. A systematic survey of the T_c s of Y-Ba-Cu-O and La-Sr-Cu-O compounds has shown that T_c is strongly correlated with hole concentration³⁻⁵, but the Hall coefficient is only weakly dependent on pressure^{6,7}. These results indicate that the large change in T_c with pressure is caused, not by a change in hole concentration, but by other factors related to changes in the interatomic distances. Here we report that the newly discovered electron-doped superconductor Nd_{1.85}Ce_{0.15}CuO_{4-δ} exhibits almost no pressure effect on T_c up to 2.5 GPa, in remarkable contrast to the hole-doped superconductor Nd_{1.3}Ce_{0.3}Sr_{0.5}CuO_{4-δ}. The Cu-O pyramids characteristic of the hole-doped compounds lose their apical oxygens to become square planes in the electron-doped materials. If the pressure effect on T_c does arise from changes in bond lengths, the difference in behaviour of the two compounds points to the involvement of the bond between copper and apical oxygen, which is missing in the electron-doped material.

The polycrystalline samples of Nd_{1.85}Ce_{0.15}CuO_{4-δ} and Nd_{1.3}Ce_{0.3}Sr_{0.5}CuO_{4-δ} studied here were prepared by the solid-state reaction technique described in ref. 8. In the Nd_{2-x}Ce_xCuO_{4-δ} solid solution, which has the Nd₂CuO₄ (T') structure (see inset of Fig. 1), superconductivity appears abruptly at a Ce composition near $x = 0.14$, with a T_c of ~ 20 K, and disappears at $x \approx 0.18$. The largest Meissner signal and the highest T_c were obtained at $x = 0.15$. The negative Hall and Seebeck coefficients observed¹ in the normal state suggest that the charge carriers are electrons, introduced by the substitution of Ce⁴⁺ for Nd³⁺. On the other hand, for Nd_{2-x-y}Ce_xSr_yCuO_{4-δ} compounds, which have the T* structure (see inset of Fig. 2), the carriers are holes and superconductivity occurs in the composition range $x = 0.15-0.2$ and $y = 0.4-0.5$ (refs 9, 10).

We measured the electrical resistivity of Nd_{1.85}Ce_{0.15}CuO_{4-δ} and Nd_{1.3}Ce_{0.3}Sr_{0.5}CuO_{4-δ} at pressures up to 2.5 GPa using a standard four-lead method. The pressure was generated by means of a Cu-Be clamp-type piston-cylinder apparatus, using a Teflon cell and a fluid pressure medium, Fluorinert No. FC70. Pressure was applied at room temperature and the apparatus was then immersed in liquid He. The pressure was determined near T_c by interpolating between the value given as load per unit area at room temperature and the value obtained by using a lead manometer at ~ 7 K. The temperature was measured with a calibrated platinum resistance thermometer in the range 20-300 K, and a germanium thermometer for temperatures of below 100 K.

Figure 1 shows the temperature dependence of resistivities obtained in Nd_{1.85}Ce_{0.15}CuO_{4-δ} near T_c at various pressures. The resistivity exhibits semiconductor behaviour, which seems not to be intrinsic but rather is due to inhomogeneities in the samples. The normal-state resistivity (R) decreases with increasing pressure at a rate of $R^{-1} dR/dP = -0.089 \text{ GPa}^{-1}$, but T_c , determined at the midpoint of the transition, is found to shift very little under pressure up to at least 2.5 GPa. This is remark-

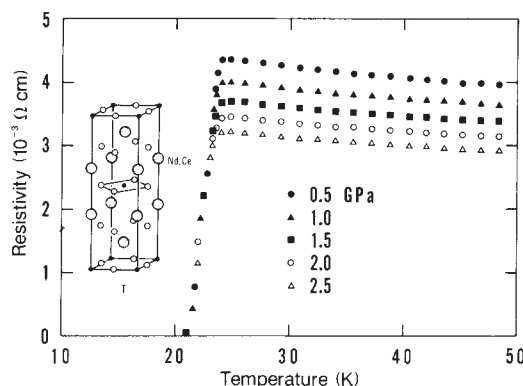


FIG. 1 The temperature dependence of the resistivity of Nd_{1.85}Ce_{0.15}CuO_{4-δ} under high pressure. Inset, the T' structure.

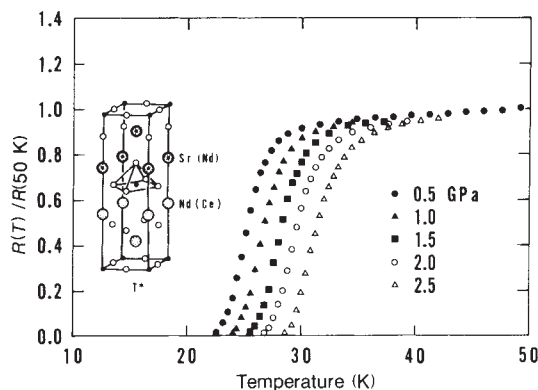


FIG. 2. The temperature dependence of the resistance of $\text{Nd}_{1.3}\text{Ce}_{0.2}\text{Sr}_{0.5}\text{CuO}_{4-\delta}$, normalized to that at 50 K under pressure. Inset, the T^* structure.

able, in view of the results obtained so far for many other copper oxide superconductors, as mentioned above. Figure 1 shows that not only the midpoint but also both the onset and zero resistivity points of the transition do not change by more than ± 0.05 K, which is a much smaller range than was obtained by Markert *et al.*¹¹.

In Fig. 2, the relative resistance (normalized at 50 K) is shown as a function of temperature for $\text{Nd}_{1.3}\text{Ce}_{0.2}\text{Sr}_{0.5}\text{CuO}_{4-\delta}$ at various pressures in the temperature range between 10 K and 50 K. At atmospheric pressure the resistivity is $\sim 3.7 \times 10^{-3} \Omega \text{ cm}$ at room temperature; with increasing pressure, it decreases at a rate of $R^{-1} dR/dP = -0.054 \text{ GPa}^{-1}$. The T_c is seen to depend strongly on pressure, in contrast to the behaviour observed for $\text{Nd}_{1.85}\text{Ce}_{0.15}\text{CuO}_{4-\delta}$. Both the transition-onset and zero-resistivity temperatures shift with pressure at the same rate as the 'midpoint' T_c .

The T_c s of these compounds are plotted as a function of pressure in Fig. 3. The pressure coefficients, dT_c/dP , for $\text{Nd}_{1.85}\text{Ce}_{0.15}\text{CuO}_{4-\delta}$ and $\text{Nd}_{1.3}\text{Ce}_{0.2}\text{Sr}_{0.5}\text{CuO}_{4-\delta}$ are 0 ± 0.05 and $3.0 \pm 0.1 \text{ K GPa}^{-1}$, respectively. The pronounced increase of T_c with increasing pressure for $\text{Nd}_{1.3}\text{Ce}_{0.2}\text{Sr}_{0.5}\text{CuO}_{4-\delta}$ is typical of hole-superconductors. The anomalous behaviour of $\text{Nd}_{1.85}\text{Ce}_{0.15}\text{CuO}_{4-\delta}$ relative to the other compounds is illustrated more clearly in Fig. 4, where we plot $d \ln T_c/dP$ against T_c . The value of $d \ln T_c/dP$ for $\text{Nd}_{1.3}\text{Ce}_{0.2}\text{Sr}_{0.5}\text{CuO}_{4-\delta}$ is located near that for oxygen-deficient $\text{YBa}_2\text{Cu}_3\text{O}_{6.3}$, both of which have almost the same T_c . The value for $\text{Nd}_{1.85}\text{Ce}_{0.15}\text{CuO}_{4-\delta}$, however, is located far below that for $\text{Nd}_{1.3}\text{Ce}_{0.2}\text{Sr}_{0.5}\text{CuO}_{4-\delta}$, despite the fact that the two compounds have almost the same T_c .

It has been suggested (ref. 2 and C.M. *et al.*, manuscript in preparation) that there might exist a general trend of increasing $d \ln T_c/dP$ values with decreasing T_c , on the basis of the proper-

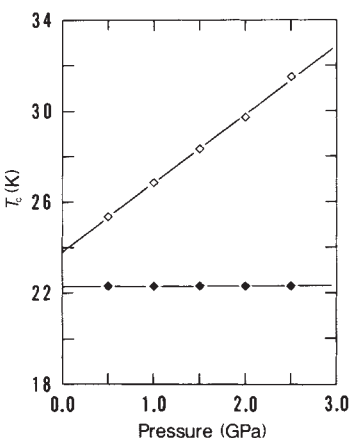


FIG. 3. The pressure dependence of the superconducting transition temperatures (T_c) of $\text{Nd}_{1.85}\text{Ce}_{0.15}\text{CuO}_{4-\delta}$ (◆) and $\text{Nd}_{1.3}\text{Ce}_{0.2}\text{Sr}_{0.5}\text{CuO}_{4-\delta}$ (◇).

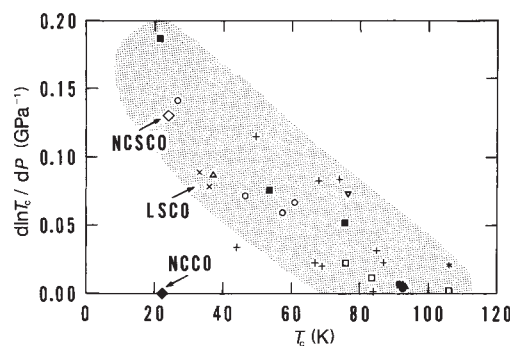


FIG. 4. The pressure coefficient of the logarithm of T_c as a function of T_c . ◆, $\text{Nd}_{1.85}\text{Ce}_{0.15}\text{CuO}_{4-\delta}$ (NCCO); ◇, $\text{Nd}_{1.3}\text{Ce}_{0.2}\text{Sr}_{0.5}\text{CuO}_{4-\delta}$ (NCSCO); ■, +, ▽; Y- or Eu-Ba-Cu(M)-O (M = Fe, Co, Ni and Zn); ○, oxygen-deficient Y-Ba-Cu-O; ●, ▲, Y- or Gd-Ba-Cu-O; □, *, Bi-Sr- or Tl-Ba-Ca-Cu-O; △, x, La-Sr-Cu-O (LSCO) (C. Murayama *et al.*, manuscript in preparation). Shaded region denotes the approximate field for all compounds except NCCO.

ties of hole-superconductors. At present there is no theory with which to compare the effect of pressure on the T_c s of the electron-doped and the hole-doped superconductors. Recently, Neumeier *et al.*¹² have shown that the pressure coefficient of T_c for $(\text{Y}_{1-x}\text{Pr}_x)\text{Ba}_2\text{Cu}_3\text{O}_{7-\delta}$ changes sign from positive to negative with increasing Pr concentration. This result was ascribed to a pronounced pressure-sensitivity of the hybridization between the localized Pr 4f state and the valence-band states. For the Nd-Ce-Cu-O and Nd-Ce-Sr-Cu-O compounds, on the other hand, Ce was confirmed, by photoemission spectroscopy, to be tetravalent, irrespective of the nature of the carriers (ref. 10 and A. Fujimori *et al.*, preprint). This implies that the Ce 4f state is not as important as the Pr state in controlling the effect of pressure, although further studies will be required to clarify this point. Rather, it seems plausible that the valence electrons donated by Ce affect the electronic structures of valence or conduction bands near the Fermi level, as suggested by A. Fujimori *et al.* (preprint).

From the structural viewpoint, it is interesting to note that $\text{Nd}_{1.85}\text{Ce}_{0.15}\text{CuO}_{4-\delta}$ has only Cu-O squares whereas in $\text{Nd}_{1.3}\text{Ce}_{0.2}\text{Sr}_{0.5}\text{CuO}_{4-\delta}$ there exist pyramidal Cu-O squares with an apical oxygen. High-pressure X-ray studies¹³ reveal that the linear compressibilities do not differ for different crystallographic axes in $\text{YBa}_2\text{Cu}_3\text{O}_{7-\delta}$ and $(\text{La}_{0.9}\text{Sr}_{0.1})_2\text{CuO}_{4-\delta}$. We suggest, therefore, that a change in the apical Cu-O distance in the pyramids may play a major part in determining the change of T_c under pressure. Indeed, those copper oxide superconductors that show the aforementioned pressure dependence of T_c (that is, those that lie within the shaded region of Fig. 4) all have Cu-O pyramids. In this respect it is desirable to investigate the effect on T_c of a uniaxial stress. □

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Determination of absolute palaeointensity using a multi-specimen procedure

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THE determination of the absolute strength of the ancient geomagnetic field from igneous rocks and other fired materials requires the original thermoremanent magnetization (palaeo-TRM) to be compared with that artificially acquired (lab-TRM) in a known field. In the Thellier approach¹, in which several sets of heatings are involved, the progressive removal (unblocking) of the palaeo-TRM is compared to the progressive acquisition (blocking-in) of the lab-TRM. But serial thermal treatments can cause significant physicochemical alteration to the magnetic carriers in a specimen (see, for example, refs 1–5), and for this reason Shaw² proposed a method employing only one thermal treatment. The peak temperature of exposure during a Shaw determination, however, must be above the highest Curie point of the sample. Here we present a new approach to the determination of palaeointensity which attempts to minimize both the number of heatings and the peak temperatures involved. It is based in principle on the Thellier method¹, in that it involves step-heating to several temperatures, but it departs dramatically from past practices in that several specimens are used for each determination. A comparison of the two methods shows that the new, multi-specimen methods may give reliable results for samples for which the Thellier method fails.

For our method, several specimens from a given site are each exposed to a set of two heatings to an individually assigned temperature. The first cooling is carried out in zero field, whereas the second cooling takes place in a chosen laboratory field³. In this way, each specimen used for a particular palaeointensity determination provides a single datum to a typical progressive plot of unblocked natural remanent magnetization (NRM) against blocked-in lab-TRM^{6,7}, and is then removed from further experimental consideration. Because of this greatly reduced exposure to thermal treatment, the procedure further promotes the use of small subsamples and hence the possibility of short exposures to elevated temperatures.

There exist particular considerations unique to the proposed procedure. First, for each subsample the plotting of unblocked NRM (palaeo-TRM) against blocked-in lab-TRM must be normalized to the initial strength of its natural remanence (NRM₀). Here, by initial we mean the primary (single-component) remanence intensity determined after any viscous remanent magnetization (VRM) has been removed through thermal

demagnetization. If perceptible VRM does not exist in all specimens involved in a given determination, no corrective measure is needed. However, if VRM cannot be considered to be a negligible component of the total NRM in all specimens, then a common, minimum temperature of thermal demagnetization need be found that effectively reduces the VRM of all specimens to insignificant values. Accordingly, each field-cooled run must then terminate at this minimum temperature, followed by further cooling to room temperature in zero field. In this way, those magnetic grains that carry VRM are isolated and removed from the palaeointensity determination.

Also unique to the method is the characteristic that any, say, linear progression of plotted data may not strictly follow the order of ascending values of peak temperature. Different specimens from the same igneous rock unit or fired material, even if found in close proximity, can possess quite distinct blocking (and unblocking) temperature spectra. When this occurs, it is possible for one specimen to unblock less palaeo-TRM (and block-in less lab-TRM) than another specimen whose assigned temperature of cooling is lower. However, such a situation in no way affects adversely the accuracy of the palaeointensity determination.

Here we use tholeiitic basalts to illustrate the proposed method; in doing so we reveal how apparently credible Thellier data can sometimes be significantly in error^{5–8}. The lavas under investigation are from a section on Molokai, Hawaii, found⁹ to record the normal-to-reverse polarity transition at the termination of the Olduvai subchron. The variation in normalized NRM intensity throughout the section suggested the possibility that particular flows, extruded near the time of completion of the polarity change, experienced strong dipole field intensities on cooling. To quantify this finding, we used the Thellier approach.

Thellier determinations for flows extruded late in the transition confirmed the strong-field behaviour suggested by the normalized NRM data; however, some flows gave surprising results which suggested dipole strengths an order of magnitude larger than today's field. Three determinations associated with one such flow (flow 1) are shown in Fig. 1. In the following, H_L is the artificial (lab) field, and H_p the absolute strength of the ancient geomagnetic field. With $H_L = 40 \mu\text{T}$ (0.40 Oe), results of $H_p = 320 \mu\text{T}$ (Fig. 1a) and $H_p = 280 \mu\text{T}$ (Fig. 1b) were obtained. We speculate that the 'noisy' appearance in both of these steep-sloped plots is due at least in part to the relative weak strength of lab-TRM acquired in a field of $40 \mu\text{T}$. To try to overcome this problem, a determination was conducted with a stronger field of $H_L = 170 \mu\text{T}$. The improved quality seen in this determination (Fig. 1c) is clear, but the result of $H_p = 350 \mu\text{T}$ is much the same as before.

The rock magnetic nature of this flow has been investigated. Thermomagnetic behaviour produced in an evacuated sample environment (Fig. 2a) indicates, not surprisingly, that the carriers are Ti-poor titanomagnetite. The degree of reproducibility

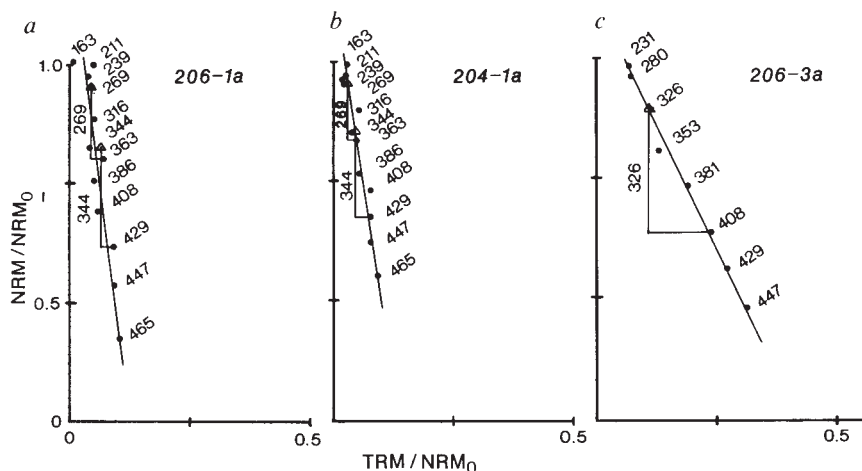


FIG. 1 Thellier determinations for flow 1 (sites 204 and 206) with temperatures indicated in °C. Furnace heatings were conducted in flowing argon; coolings took place in ambient fields of $<20 \text{ nT}$. Laboratory fields H_L and measured palaeointensity H_p were: a, $H_L = 40 \mu\text{T}$, $H_p = 320 \mu\text{T}$; b, $H_L = 40 \mu\text{T}$, $H_p = 280 \mu\text{T}$; c, $H_L = 170 \mu\text{T}$, $H_p = 350 \mu\text{T}$. Partial TRM checks¹ of reliability are also shown.

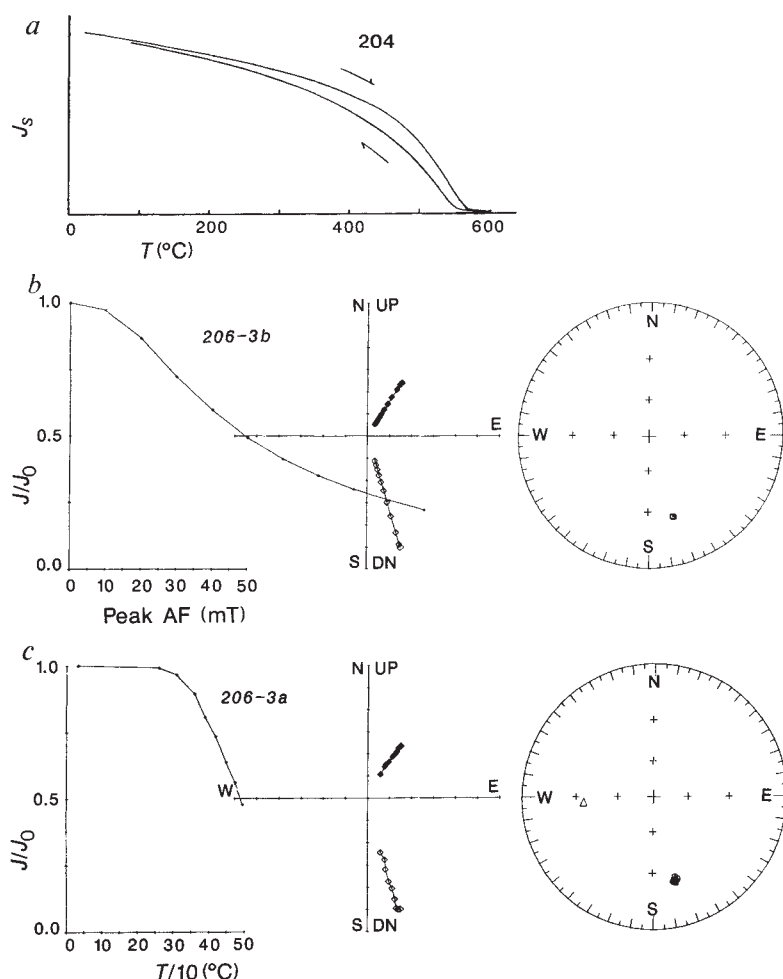


FIG. 2 Rock magnetic properties for flow 1. *a*, Thermomagnetic heating and cooling curves. J_s is the saturation magnetization. *b*, *c*, Normalized intensity J/J_0 , orthogonal directional plots (solid (open) symbols: up-down (N-S) versus E-W), and stereographic plots (solid (open) symbols: lower (upper) hemisphere) during (b), AF demagnetization, and (c), the thermal demagnetization portion of the Thellier run shown in Fig. 1c. The triangle in the stereographic plot indicates the direction of H_L during field-coolings.

between heating and cooling curves suggests that physicochemical alteration is not a major factor during heat treatment even to 600 °C. In addition, very stable palaeodirectional behaviour is seen during alternating field (AF) demagnetization (Fig. 2b). These data indicate that the magnetic carriers are fine-grained, containing a single-component, reverse-polarity remanence direction and, apparently, an imperceptible normal polarity (VRM) overprint. In Fig. 2c we show the thermal demagnetization part (that is, measurements after each zero-field cooling) of the $H_L = 170 \mu\text{T}$ run (Fig. 1c). Any progressive rotation of the remanence vector toward the direction of the applied field (see Fig. 2c) would suggest that alteration had occurred, and that a chemical remanent magnetization (CRM) had been induced during the second, field-cooled runs. However, there is no visual indication that CRM was acquired throughout the course of the experiment.

These data seem to be consistent and to support the reliability of the Thellier results shown in Fig. 1. However, we find that these findings of a particularly strong palaeofield conflict with results from the multi-specimen approach described below, and we are led to suggest that the Thellier results are spurious, caused by progressive physicochemical alteration.

Subsamples for the multi-specimen approach were cut from a single 2.4-cm-diameter basalt core from flow 1 such that each 2.5-cm height was quartered (see Fig. 3). For comparison with the Thellier results plotted in Fig. 1c, we again used a field of $H_L = 170 \mu\text{T}$. Results are shown in Fig. 3. In contrast to the simple linearity of the Thellier plot, these data fall along what seem to be two distinct linear segments. More specifically, data corresponding to heating temperatures below (above) about 300 °C are found along the upper (lower) linear segment.

Some Hawaiian basalts have been found to show atypical,

broken-segment Thellier behaviour^{10,11}. For our case, however, the Thellier results (Fig. 1) are in striking contrast to those obtained by the new method. Indeed, the multi-specimen data (Fig. 3) suggest that heating to ~300 °C severely alters the blocking-temperature spectrum of magnetic carriers in this par-

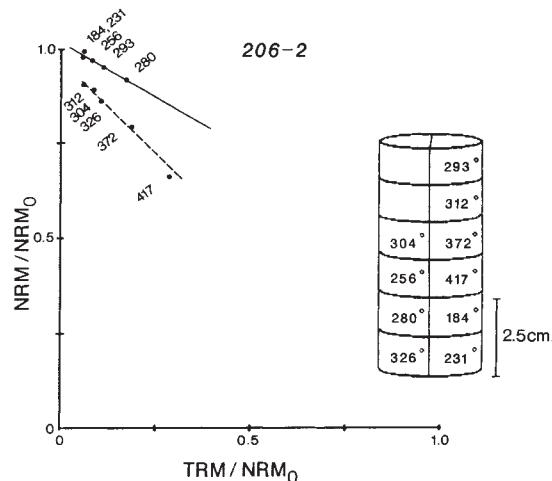


FIG. 3 Multi-specimen determination for flow 1, with temperatures indicated in °C. Each unblocked palaeo-TRM and blocked-in lab-TRM datum are normalized to the total NRM intensity of the respective specimen. The laboratory field, $H_L = 170 \mu\text{T}$, is the same as that applied during the Thellier run shown in Fig. 1c. The schematic drawing on the right depicts the assigned temperature of each subsample, chosen in an essentially random fashion. The palaeointensity, $H_p = 100 \mu\text{T}$, was determined from the upper (that is, lower-temperature) linear segment. See text for further explanation.

ticular flow. Indeed, specimens heated to higher temperatures seem to lose much of their ability to acquire palaeo-TRM, when compared with specimens heated to temperatures below 300 °C. We suspect that as the peak temperature approaches 300 °C, an increasing fraction of magnetic grains alter in such a manner that their blocking temperatures are raised significantly. Thus, those grains responsible for the acquisition of laboratory palaeo-TRM at lower temperatures during the (second) field-cooling may lose this capability just before being cooled from a higher temperature.

Adopting this as a working hypothesis enables us to explain the significantly steepened, single-segment Thellier result (Fig. 1c): although the thermal treatment of subsamples employed in the multi-specimen determination may not be sufficient to produce detectable physicochemical alteration until the assigned temperature approaches 300 °C, the cumulative effect of stepwise heatings associated with the Thellier method may indeed cause significant sample degradation which is observable at lower temperatures. A single-segment, spuriously steepened plot may be roughly explained if we take as our starting point the multi-specimen data (Fig. 3) and apply with each successive temperature step up to 300 °C a progressively reduced intensity of blocked-in lab-TRM.

Thus it seems that the true palaeointensity for basalt flow 1 is better determined from the slope of the upper (that is, lower-temperature) segment of Fig. 3, that is, from data associated with specimens that are least likely to have been altered during laboratory heating. A palaeofield strength $H_p \approx 100 \mu\text{T}$ is determined from this reasonably well defined linear segment, which is only 30% of the Thellier result.

In contrast to flow 1, the application of both methods to the adjacent flow (flow 2) gives single, linear segments over a considerable range of temperature (Fig. 4). Subjected to the same laboratory field strength, $H_L = 170 \mu\text{T}$, the slopes are found to be essentially identical (associated with a palaeointensity $H_p = 45 \mu\text{T}$). Clearly, the specimen degradation which occurred during thermal treatment of flow 1 did not occur during these determinations. Moreover, because of the very different heating procedures involved with the two methods, we argue that physicochemical alteration may be confidently ruled out as a source of error and that the palaeointensity determination for flow 2 is very reliable.

Thus we obtained reliable palaeointensity determinations for both basalt flows (1 and 2) by using the multi-specimen method. Clearly, the Thellier approach fails for the flow 1 samples

considered here, even though the palaeointensity data, and other relevant rock-magnetic properties, give the impression of credibility. Why such 'ideal' basaltic rocks are apparently incapable of providing reliable Thellier results is now under investigation. Continued testing and use of the new method will ultimately determine the extent of its utility to archaeomagnetic and palaeomagnetic studies. □

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Oxidation of aromatic contaminants coupled to microbial iron reduction

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THE contamination of sub-surface water supplies with aromatic compounds is a significant environmental concern^{1,2}. As these contaminated sub-surface environments are generally anaerobic, the microbial oxidation of aromatic compounds coupled to nitrate reduction, sulphate reduction and methane production has been studied intensively^{1–7}. In addition, geochemical evidence suggests that Fe(III) can be an important electron acceptor for the oxidation of aromatic compounds in anaerobic groundwater. Until now, only abiological mechanisms for the oxidation of aromatic compounds with Fe(III) have been reported^{8–12}. Here we show that in aquatic sediments, microbial activity is necessary for the oxidation of model aromatic compounds coupled to Fe(III) reduction. Furthermore, a pure culture of the Fe(III)-reducing bacterium GS-15 can obtain energy for growth by oxidizing benzoate, toluene, phenol or *p*-cresol with Fe(III) as the sole electron acceptor. These results extend the known physiological capabilities of Fe(III)-reducing organisms and provide the first example of an organism of any type which can oxidize an aromatic hydrocarbon anaerobically.

At the onset of anaerobic conditions, Fe(III) is the most abundant potential electron acceptor for organic-matter oxidation in most fresh-water sedimentary environments^{13,14}. The accumulation of Fe(II) during the anaerobic oxidation of organic matter in numerous pristine and contaminated aquifers further suggests that Fe(III) is a potential electron acceptor in a wide variety of sub-surface environments^{15,16}. An example of the potential role of Fe(III) in oxidizing contaminants in groundwater was observed in a glacial-outwash aquifer located in Bemidji, Minnesota. The aquifer was contaminated by crude oil from a break in a pipeline in 1979¹⁷. A plume of anaerobic groundwater developed down gradient from the oil body that floats on the water table. Benzene and alkylbenzenes are leaching

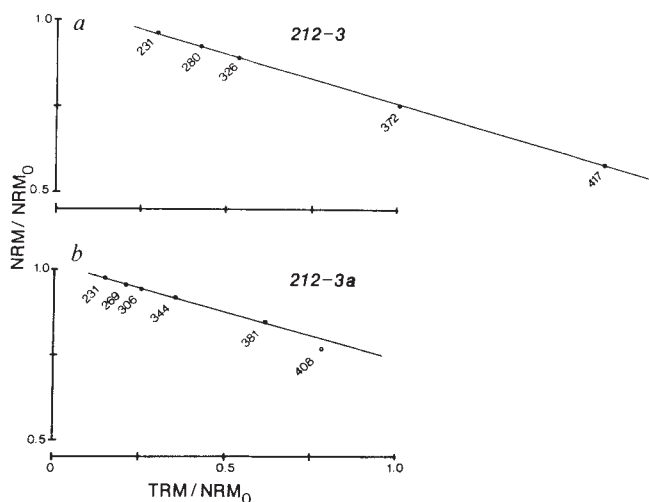


FIG. 4 Determinations by *a*, the multi-specimen and *b*, the Thellier methods, for flow 2 (site 212), employing the same $H_L (=170 \mu\text{T})$. The palaeointensity $H_p = 45 \mu\text{T}$ is the same for both determinations. (Note that the Thellier data show signs of alteration effects above 400 °C, but the multi-specimen data do not.)

into the anaerobic groundwater but their concentrations decrease rapidly along the groundwater flow path^{18,19}. Isomeric alkylbenzenes decrease in concentration at different rates in the anaerobic plume, indicating that the aromatic contaminants are not removed by physical-chemical processes such as adsorption, volatilization or dilution^{18,19}. In 1984, initial studies on the anaerobic groundwater at a site, Bemidji A, near the edge of the oil lens indicated that the dissolved inorganic carbon in the contaminated groundwater was isotopically lighter than that in the native groundwater (Fig. 1). This suggested that the isotopically light contaminant hydrocarbons ($\delta^{13}\text{C}$ of -28%) were being converted to carbon dioxide.

Fe(III) appeared to be an important electron acceptor for the anaerobic oxidation of the aromatic hydrocarbons to carbon dioxide. Fe(II), the reduced product of the reduction of Fe(III), accumulated over time in the contaminated groundwater at Bemidji A but was undetectable in the native groundwater at an uncontaminated background site (Fig. 1). By 1988 cores of sediments from Bemidji A contained only 4 mmol of oxalate-extractable Fe(III) (ref. 20) per kg of sediment. Sediments from the uncontaminated background site contained four times as much Fe(III). The native groundwater contained too little nitrate or sulphate (less than $20\ \mu\text{M}$, as determined by ion chromatography) for these electron acceptors to be significant anaerobic oxidants of the contaminants.

By 1987 it appeared that methane production had also become a terminal electron-accepting process at Bemidji A (Fig. 1). This transition from Fe(III) reduction as the predominant terminal electron-accepting process to simultaneous Fe(III) reduction and methane production is typically observed in fresh-water sediments as the availability of Fe(III) for microbial reduction diminishes^{21,22}. Although the inorganic carbon produced during this period was still isotopically lighter than that in the native groundwater, it became progressively heavier, presumably as a result of methanogenic bacteria preferentially reducing light carbon dioxide to methane²³.

The increasing importance of methane production at Bemidji A meant that studies on the oxidation of aromatic compounds with Fe(III) as the only electron acceptor were no longer possible at this site. Thus, in 1988 a new site, designated Bemidji B, was sampled with a split-spoon corer to obtain sub-surface sediments in which Fe(III) reduction was still the predominant terminal electron-accepting process. Bemidji B, which was located only 5 m further along the flow path of the anaerobic plume than Bemidji A, was still found to contain ample oxalate-extractable Fe(III), (17 mmol per kg of sediment), to support Fe(III) reduction. Using strict anaerobic techniques²⁴, Bemidji B sediments (20 ml) were transferred under $\text{N}_2\text{-CO}_2$ (at a ratio of 93:7) into

serum bottles (30 ml capacity) which were then sealed with butyl rubber stoppers. Fe(III) reduction and methane production were monitored as previously described²⁴. After a two-month incubation at the *in situ* temperature of 9°C , 1.5 mmol of HCl-extractable Fe(II)²⁴ had accumulated per kg of sediment and no methane had been produced. This result indicated that Fe(III) reduction was the predominant terminal electron-accepting process for organic-matter oxidation in the sediments collected from Bemidji B.

To determine whether the apparent oxidation of aromatic hydrocarbons to carbon dioxide during the reduction of Fe(III) in sub-surface sediments was the result of abiotic or biotic reactions, Bemidji B sediments (25 ml) were anaerobically placed into serum bottles (50 ml) which were then completely filled with anaerobic groundwater from Bemidji B. The bottles were sealed without a headspace with Teflon-lined butyl rubber stoppers. Toluene was added with a microsyringe to provide an initial concentration of $600\ \mu\text{M}$. Toluene concentrations were measured by extracting the sediment slurries with pentane and quantifying the toluene in the extract by flame ionization-gas chromatography. After 45 days of incubation, less than 2% of the toluene remained. However, in sediments in which the microorganisms had been killed with heat (121°C for 1 h) before incubation, there was no loss of toluene and no Fe(III) reduction. These results suggest that the disappearance of toluene during the reduction of Fe(III) was microbially mediated.

To examine further the potential role of microorganisms in the oxidation of aromatic compounds coupled to the reduction of Fe(III), the oxidation of benzoate in the Fe(III)-reducing sediments was investigated. Benzoate is most frequently used as a model compound for aromatic decomposition studies³ and the accumulation of benzoate and methylbenzoate compounds in the contaminated groundwaters of the Bemidji aquifer suggest that benzoate is an important intermediate in the oxidation of monoaromatic hydrocarbons under Fe(III)-reducing conditions¹⁹. When the Bemidji B sediments in which Fe(III) reduction was the terminal electron-accepting process were injected with uniformly ring-labelled [^{14}C]benzoate under anaerobic

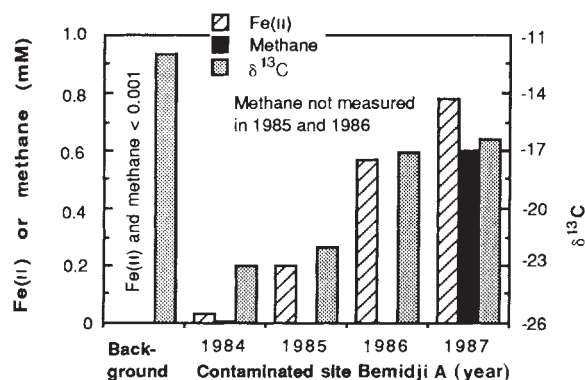


FIG. 1 Concentrations of Fe(II) and methane and the $\delta^{13}\text{C}$ (relative to the PDB standard) of the inorganic carbon dissolved in the anaerobic groundwater at site Bemidji A, and in the aerobic native groundwater at a nearby uncontaminated background site. Groundwater was sampled with a peristaltic pump and dissolved Fe(II) and methane were determined with standard geochemical techniques as previously described²³.

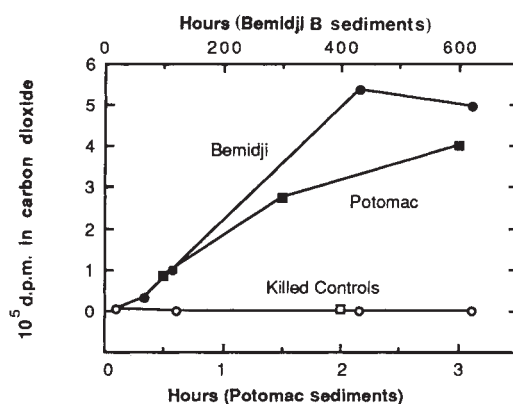


FIG. 2 Production of [^{14}C]carbon dioxide from uniformly ring-labelled [^{14}C]benzoate in sediments with Fe(III) reduction as the terminal electron-accepting process. Bemidji sub-surface sediments were from site Bemidji B, and Potomac River sediments were surface fresh-water sediments in which it was known²² that Fe(III) reduction was the terminal electron-accepting process. [^{14}C]benzoate (0.1 ml containing 0.35 μCi of 120 mCi per mmol benzoate) was anaerobically added to 10 ml of sediment that was incubated under $\text{N}_2\text{-CO}_2$ (in a ratio of 93:7) in anaerobic pressure tubes which were sealed with butyl rubber stoppers. At the designated times 1 ml of $2.5\ \text{M}\ \text{H}_2\text{SO}_4$ was added with a syringe and needle. N_2 was flushed through the tubes and into a series of vials containing $1\ \text{M}\ \text{NaOH}$ to trap $^{14}\text{CO}_2$. A scintillation counter was used to determine $^{14}\text{CO}_2$. For Bemidji sediments, killed controls were treated with heat (121°C for 1 h) before the addition of [^{14}C]benzoate. For Potomac river sediments, killed controls received additions of glutaraldehyde (2.5% final concentration) before the addition of [^{14}C]benzoate.

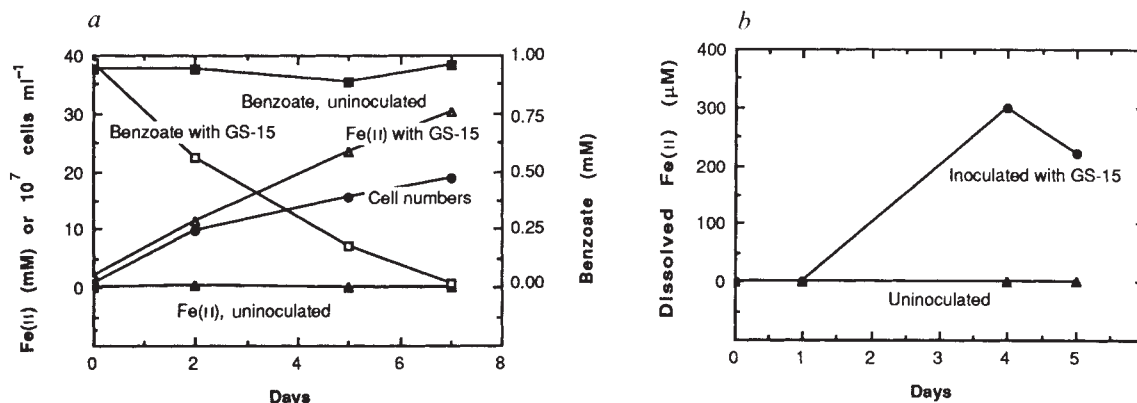
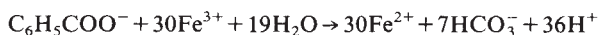


FIG. 3 Benzoate metabolism coupled to reduction of Fe(III) by the dissimilatory Fe(III)-reducing bacterium, GS-15. *a*, Growth in a medium²⁸ with benzoate as the sole electron donor and Fe(III)-citrate as the sole electron acceptor. Benzoate was determined with HPLC. Fe(II) and cell numbers were quantified with ferrozine and by microscopy, respectively as previously described²⁸. *b*,

Production of dissolved Fe(II) when subsurface sediments (10 ml) from an uncontaminated portion of the Bemidji aquifer were mixed with an equal volume of medium²⁸ with 1.5 mM benzoate as the sole electron donor. The Fe(III) in the sediments was the only electron acceptor provided.

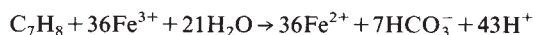
conditions, the [¹⁴C]benzoate was oxidized to ¹⁴CO₂, but only if biological activity was not inhibited (Fig. 2). A similar biologically dependent oxidation of [¹⁴C]benzoate was observed in fresh-water sediments from the Potomac River in which Fe(III) reduction was the predominant terminal electron-accepting process (Fig. 2).

It has long been recognized that microbial activity can enhance the oxidation of organic compounds coupled to the reduction of Fe(III)^{25,26}. However, the ability of Fe(III)-reducing microorganisms to directly link the complete oxidation of organic compounds to Fe(III) reduction has only recently been recognized²⁷⁻³⁰. The dissimilatory iron-reducing bacterium, strain GS-15, was previously known to obtain energy for growth by oxidizing short-chain fatty acids or ethanol to carbon dioxide with amorphous Fe(III) oxide or Fe(III) citrate as the sole electron acceptor²⁸. However, after an adaptation period (~1 month) GS-15 grew vigorously with benzoate and either of the Fe(III) forms. Growth was associated with the metabolism of benzoate and the concomitant reduction of Fe(III) to Fe(II) (Fig. 3*a*). The stoichiometry of benzoate metabolism (Fig. 3*a*) was consistent with the reaction



With benzoate as the sole electron donor, GS-15 could also reduce the Fe(III) in the sub-surface sediments at Bemidji (Fig. 3*b*). This demonstrates that there are microorganisms that can couple the oxidation of aromatic compounds to the reduction of naturally occurring Fe(III) oxides.

GS-15 also reduced Fe(III) in a medium²⁸ with toluene as the sole electron donor and amorphous Fe(III) oxide as the sole electron acceptor. The volatility of toluene and its tendency to be absorbed into the butyl rubber stoppers prevented quantitative measurements of toluene uptake. However, carbon dioxide production and Fe(III) reduction were measured as previously described²⁸ after GS-15 was cultured through three successive transfers (10% inoculum) on toluene-Fe(III) medium. In a medium containing no more than 1 mmol l⁻¹ toluene as the sole electron donor, and amorphous Fe(III) oxide as the sole electron acceptor, GS-15 produced 1.6 mM carbon dioxide and 9.3 mM Fe(II) during a two week incubation. No extracellular intermediates were detectable with high-performance liquid chromatography. These results are consistent with the reaction



Although the oxidation of aromatic hydrocarbons has recently been documented in anaerobic systems containing mixed microbial populations with nitrate reduction or methane production as the terminal electron-accepting process^{2,5-7}, GS-15 is the first

organism in pure culture known to oxidize an aromatic hydrocarbon under anaerobic conditions.

GS-15 was also capable of oxidizing two other common aromatic contaminants, phenol and *p*-cresol, at initial concentrations of 1 mM, with amorphous Fe(III) oxide as the electron acceptor. GS-15 could not oxidize other model aromatic compounds like phthalic acid, nicotinic acid, ferulic acid or syringic acid. However, enrichment cultures which oxidize these compounds with the reduction of Fe(III) were readily obtained by inoculating sediments into an anaerobic medium (pH 6.7) (ref. 28) that contained one of the aromatics as the sole electron donor and amorphous Fe(III) oxide as the sole electron acceptor. None of the aromatic compounds metabolized by GS-15, or the enrichment cultures reduced Fe(III), in the absence of microorganisms. In testing a wide variety of aromatic compounds we have found only one, catechol, which abiotically reduces amorphous Fe(III) oxide at the near-neutral pH values typical of most groundwater environments. Given the widespread availability of Fe(III) as a potential electron acceptor in subsurface environments¹³⁻¹⁶, it seems likely that microbial oxidation of aromatic contaminants, coupled to dissimilatory Fe(III) reduction, may be capable of removing significant quantities of aromatic compounds from many contaminated aquifers. □

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Genetic segregation and the maintenance of sexual reproduction

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SEXUAL reproduction confronts evolutionary biology with a paradox: other things being equal, an asexual (all-female) population will have twice the reproductive potential of a competing sexual population and therefore should rapidly drive the sexual population to extinction^{1,2}. Thus, the persistence of sexual reproduction in most life forms implies a compensatory advantage to sexual reproduction. Work on this problem has emphasized the evolutionary advantages produced by the genetic recombination that accompanies sexual reproduction¹⁻⁷. Here we show that genetic segregation produces an advantage to sexual reproduction even in the absence of an advantage from recombination. Segregation in a diploid sexual population allows selection to carry a single advantageous mutation to a homozygous state, whereas two separate mutations are required in a parthenogenetic population. The complete fixation of advantageous mutations is thus delayed in a heterozygous state in asexual populations. Calculation of the selective load incurred suggests that it may offset the intrinsic twofold reproductive advantage of asexual reproduction and maintain sexual reproduction in diploid populations.

To determine the magnitude of the genetic load in an asexual population caused by loci caught in the heterozygous state, consider the fate of advantageous mutations arising at L homozygous wild-type loci, assuming for simplicity that L is constant. Let the mutant heterozygotes be intermediate between the wild-type and mutant homozygotes with fitness $1 + hs$, 1 and $1 + s$ respectively, where s and h are positive and <1 . We also assume that no more than one advantageous mutation will be spreading through the population at any time to ensure that there is no advantage to sexual reproduction derived from recombination.

The probabilities that equivalent advantageous mutations at homozygous wild-type loci spread in an asexual and in a sexual

population are approximately equal as segregation does not alter the relative fitnesses of the wild-type homozygote and the mutant heterozygote. If a mutation spreads, however, it will reach fixation for the homozygote in a sexual population via segregation but only fixation for the heterozygote in an asexual population (Fig. 1). The rate at which loci become fixed in the heterozygous state in an asexual population is $2\mu NLP_0$, where μ is the per allele mutation rate to advantageous alleles, N the population size and P_0 the probability that a single advantageous mutation at a homozygous wild-type locus spreads. To be converted completely to the advantageous homozygote, a second mutation must occur in a heterozygote and then spread. The rate at which loci fixed as heterozygotes are converted to the advantageous homozygotes in an asexual population is $\mu Nn P_1$, where P_1 is the probability that a mutation converting a heterozygote to an advantageous homozygote spreads through the population and n is the number of loci fixed in the heterozygous state. Thus, the rate of change in the number of loci fixed in the heterozygous state in an asexual population is $dn/dt = 2\mu NLP_0 - \mu Nn P_1$. From classical theory (ref. 3), $P_0 \approx 2hs$ and $P_1 \approx 2s(1-h)/(1+hs)$ when $s \ll 1$ and $Ns \gg 1$. At equilibrium, therefore, the number of loci in this state is

$$\hat{n} \approx \frac{2Lh(1+hs)}{1-h} \quad (1)$$

These heterozygous loci create a genetic load for an asexual population because equivalent loci go on to fixation for the advantageous homozygote in a sexual population (Fig. 1). To calculate the magnitude of fitness loss for an asexual population we assume that the fitness effects of loci are simply multiplicative. The effect of this genetic load is to give a sexual population a fitness of

$$W_s = \left(\frac{1+s}{1+hs} \right)^n \quad (2)$$

relative to an equivalent asexual population. When $W_s > 2$, this advantage to the sexual population more than compensates for its intrinsic twofold reproductive deficit.

Equilibrium values of W_s as a function of s and L are shown in Table 1. Equation (2) and Table 1 show that larger values of s and L produce increases in the equilibrium value of W_s and so give an increasing advantage to sexual reproduction. Even moderate selection coefficients and numbers of mutable loci are sufficient to maintain sexual reproduction (Table 1). The equilibrium for W_s is unaffected by the mutation rate μ or the population size N , but the rate at which loci fixed in the heterozy-

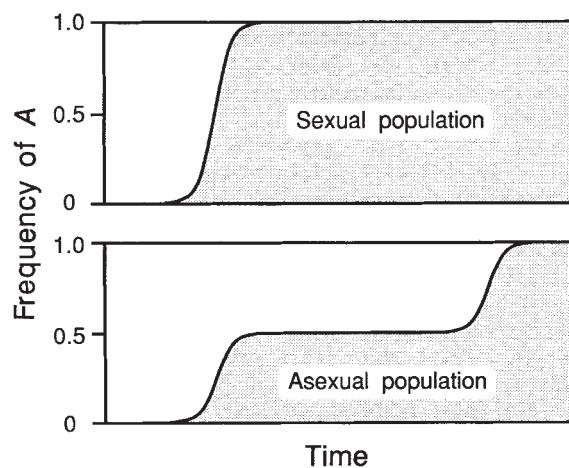


FIG. 1 Schematic illustration of the fates of an advantageous mutation A spreading through sexual and asexual diploid populations. The locus in the asexual population is fixed in a heterozygous state until a mutation appears at the second allele. The second mutation takes on average twice as long to appear, as only one allele is available to mutate in the heterozygote.

TABLE 1 Equilibrium values of W_s , the fitness of a sexual population relative to an asexual population

s	L	10	50	100	500	1,000
0.001	1.0	1.1	1.1	1.6	2.7	
0.005	1.1	1.3	1.6	1.2×10^1	1.5×10^2	
0.01	1.1	1.6	2.7	1.5×10^2	2.1×10^4	
0.05	1.6	1.2×10^1	1.4×10^2	5.3×10^{10}	2.8×10^{21}	
0.1	2.7	1.3×10^2	1.7×10^4	1.6×10^{21}	2.7×10^{42}	

Values below the heavy line exceed 2, indicating a net fitness advantage to the sexual population. Calculations assume $h=1/2$.

gous state will accumulate in a newly arisen parthenogenetic population is proportional to these two parameters. To give an example of the timescales involved, a newly arisen asexual population takes $\approx 10^6$ generations to accumulate a genetic load that more than offsets its twofold reproductive advantage, when compared to an equivalent sexual population, if $s = 10^{-2}$, $h = 1/2$, $N = 10^5$, $\mu = 10^{-9}$ and $L = 10^2$.

We now return to several of our assumptions. First, we have shown that segregation can benefit a sexual population in precisely the situation where recombination has no effect by assuming that, at most, a single advantageous mutation is spreading through the population at one time (roughly when $4NL\mu \ln(2Ns) \ll 1$ if $h \approx 1/2$). An advantage gained through recombination can supplement the advantage gained through segregation if advantageous mutations occur frequently enough so that several are simultaneously spreading at different loci²⁻⁶. The segregation advantage will largely be lost if the mutation rate is very high (roughly when $2N\mu \ln(2Ns)$ is not much less than 1) because advantageous homozygotes appear in asexual populations rapidly enough so that loci are not fixed in the heterozygous state. Second, we have assumed that there is a constant pool of L loci in wild-type homozygous state at which advantageous mutations can occur. More realistically, the number of such loci will fluctuate as these loci mutate (decreasing

L), and as new loci are added to the pool, for example through deterioration of the environment (increasing L). A constant value of L can be thought of as the average size of the pool produced by these opposing processes. Third, the effect we have described depends on the spread of new advantageous mutations at the L loci. Segregation can produce an advantage to sexual reproduction even when these loci, at which mutation is the limiting step for evolution, represent only a small fraction of the genome. Fourth, the mechanism described here functions only when heterozygotes have intermediate fitness. There will be a disadvantage to segregation and hence to sexual reproduction if mutations have highest fitness when heterozygous. Finally, we have assumed a diploid genetic system. The mechanism described here cannot explain the origin or maintenance of sexual reproduction in species that are haploid for a large part of their lives, such as some protists, fungi, algae and bryophytes. It can, however, explain the maintenance of sex in polyploids.

Muller⁴ expressed the view of many evolutionary biologists regarding sexual reproduction when he said that "of [its] two major features, segregation and recombination, only recombination is in itself of evolutionary value". The very simple consideration we raise here shows that, on the contrary, segregation is of consequence for diploid organisms. Segregation may in fact contribute substantially to repaying the cost of meiosis. □

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A potential basis for the thrombotic risks associated with lipoprotein(a)

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LIPOPROTEIN(a) (Lp(a)) has been strongly linked with atherosclerosis and is an independent risk factor for myocardial infarction¹⁻⁶. Distinguishing Lp(a) from other low-density lipoprotein particles is its content of a unique apoprotein, apo(a)⁷⁻⁹. The recently described sequence of apo(a) indicates a remarkable homology with plasminogen^{10,11}, the zymogen of the primary thrombolytic enzyme, plasmin. Lp(a) may contain 37 or more disulphide-looped kringle structures, which are 75-85% identical to the fourth kringle of plasminogen. Plasminogen receptors are widely distributed on blood cells¹² and are present at extremely high density on endothelial cells^{13,14}. These receptors promote thrombolysis by accelerating plasminogen activation^{13,15} and protecting plasmin from inhibition^{12,16}. If, by molecular mimicry, Lp(a) competes with plasminogen for receptors, then thrombolysis would be inhibited and thrombosis promoted. Here we provide support for such a mechanism being responsible for the thrombotic risks associated with elevated Lp(a) by demonstrating that Lp(a) inhibits plasminogen binding to cells.

To test our hypothesis, we determined whether isolated Lp(a) competes with plasminogen for cellular binding sites. Human umbilical vein endothelial cells (HUVEC) and human monocyte-like U937 cells were used as models. The selection

of these cells was based on their central role in atherogenesis and their extensively characterized interactions with plasminogen^{13,14,16-18}. Glu-plasminogen, which is the native circulating form of the zymogen, was isolated from fresh human plasma under conditions that minimize degradation^{19,20} or activation, and then radioiodinated. Selected preparations were further purified by filtration on Ultrogel AcA44 to remove trace Lp(a) contamination. Lp(a) was isolated from fresh human plasma using flotation centrifugation followed by affinity chromatography on lysine-Sepharose^{9,11}. We did not detect any cross-contamination of plasminogen and Lp(a) preparations by sensitive immunoassay²¹ (<1%). The capacity of non-labelled Lp(a) and plasminogen to inhibit the interaction of the ¹²⁵I-labelled plasminogen with the cells was assessed under equilibrium conditions.

Lp(a) inhibited the binding of ¹²⁵I-labelled plasminogen to U937 cells and the inhibition curve produced by Lp(a) was superimposable on that produced by non-labelled plasminogen (Fig. 1), indicating that Lp(a) and plasminogen could be interacting with the ¹²⁵I-labelled plasminogen binding sites with comparable affinity. The average concentration for 50% inhibi-

TABLE 1 Competition for plasminogen receptors by kringle 4-containing molecules

Cell type	Plasminogen	IC ₅₀ , μ M Lp(a)	EDP-II
U937 monocytoid cells	0.65 $\pm$ 0.37	0.69 $\pm$ 0.4	45
Umbilical vein endothelial cells	1.8 $\pm$ 1.1	0.13 $\pm$ 0.03	43

The IC₅₀ values for plasminogen and Lp(a) are derived from experiments such as those shown in Figs 1 and 2 and represent the means $\pm$ s.d. of three to four separate determinations, with each experiment containing duplicate or triplicate points. EDP-II is the elastase degradation product of plasminogen and corresponds to Val(354)-Ala(439), the kringle 4 region of the molecule. The IC₅₀ values for this fragment corroborate previously published results²⁰ with other cell types.

tion (IC_{50}) of Lp(a) for ^{125}I -labelled plasminogen binding was not significantly different ($P > 0.05$) from that of non-labelled plasminogen (Table 1). Maximal inhibition by Lp(a) and plasminogen were comparable (see Fig. 1), indicating that Lp(a) is occupying the same sites that bind plasminogen. The inhibition by Lp(a) was not due to cell lysis, or to loss of viability: $>95\%$ cell recovery and trypan-blue dye exclusion were observed after a 60-min incubation of the cells with $1.4 \mu M$ Lp(a). Degradation of the apo(a) was negligible ($<1\%$), as determined by densitometric scans of immunoblotted gel transfers⁹, suggesting that the observed inhibition did not depend on proteolysis of Lp(a). That the inhibition of plasminogen binding by Lp(a) was specific was shown by similar concentrations of apo(a)-free low-density lipoprotein (LDL) having no effect on ligand binding (Fig. 1). Finally, the inhibitory effects of Lp(a) observed at $37^\circ C$ (Fig. 1) were also seen at $4^\circ C$, a temperature at which internalization of bound ligands is minimized.

We also assessed the capacity of Lp(a) to inhibit the binding of ^{125}I -labelled plasminogen to monolayer cultures of HUVEC. Lp(a) competed with ^{125}I -labelled plasminogen in a dose-dependent manner and produced complete inhibition at high concentrations (Fig. 2). As summarized in Table 1, Lp(a) was ~ 14 -fold more potent than plasminogen in inhibiting ligand binding. As the extent of plasminogen binding with different endothelial cell cultures varies considerably^{13,14}, and the sample size was small, this difference was not highly significant ($P = 0.12$). In controls, microscopic examination showed that Lp(a) preparations did not disrupt the endothelial cell monolayer; immunoblotting analyses indicated that Lp(a) was not degraded in the presence of the cells, and LDL failed to inhibit plasminogen binding.

Considering the extensive repetition of the kringle 4-like structures in Lp(a), we compared the inhibitory potency of Lp(a) and kringle 4 (isolated as an elastase degradation product (EDP-II) of plasminogen²²). Integrated and isolated kringle 4 have similar conformational²³, immunochemical^{24,25} and functional properties²⁶. Based on IC_{50} values (Table 1), Lp(a) was 100 to 300 times more potent an inhibitor of plasminogen binding than

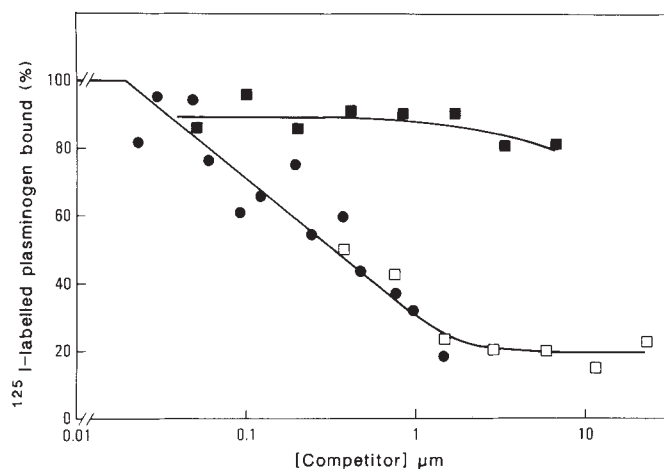


FIG. 1 Effect of Lp(a) on the binding of plasminogen to the human monocytoid U937 cells. U937 cells were washed and suspended at 3×10^6 per ml in Hanks' balanced salt solution containing 0.1% bovine serum albumin and buffered at pH 7.4 with 0.05 M HEPES. Lp(a) (●); apo(a)-free LDL (■), purified by FPLC as described²⁷, and non-labelled plasminogen (□) were added at the same time as $0.2 \mu M$ ^{125}I -plasminogen (specific activity, $0.7 \mu Ci \mu g^{-1}$) in a total volume of 200 μl . Binding was measured after 60 min at $37^\circ C$ by separation of bound from free ligand after centrifugation through sucrose^{14,15,18}. The data points were calculated as the inhibition of specific binding of ^{125}I -labelled plasminogen. Specific binding was defined as the component of total ligand binding that was inhibitable by 0.2 M 6-aminohexanoic acid (a lysine analogue that interferes with plasminogen binding to cells^{14,15,18} by interacting with the lysine binding sites of plasminogen); it represented 94% of the total binding.

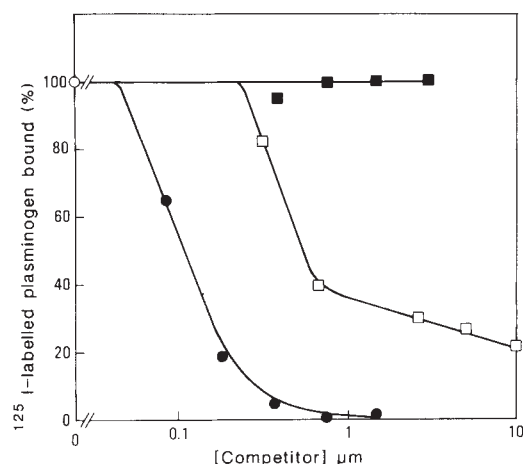


FIG. 2 Effect of Lp(a) on the binding of plasminogen to human umbilical vein endothelial cells. Binding assays were performed with second passage endothelial cells grown to confluence in 24-well Costar tissue culture plates¹⁴ in the buffer used in the experiment shown in Fig. 1, but supplemented with 2.4 mM $CaCl_2$ and 0.8 mM $MgSO_4$. The cells were incubated with ^{125}I -labelled plasminogen ($0.2 \mu M$) and non-labelled Lp(a) (●), apo(a)-free LDL (■) or plasminogen (□) for 60 min at $37^\circ C$. After aspirating the fluid, the cultures were washed three times, and the cell-associated radioactivity was extracted with 1% SDS. Specific binding was measured as described in Fig. 1.

the isolated kringle. Thus, the capacity of Lp(a) to compete with plasminogen for cellular binding sites must reside in concatenation of kringle 4-like structures within Lp(a), or be attributable to other homologies between the molecules¹⁰ such as the kringle 5 light-chain region of Lp(a).

Lp(a) levels >0.25 – 0.4 mg ml^{-1} are associated with a significantly higher incidence of myocardial infarction^{5,6}. If an IC_{50} of $\sim 0.3 \mu M$ (average of endothelial cell and U937 cell IC_{50} values) approximates the binding constant (K_d) of Lp(a) for plasminogen binding sites, and assuming an average K_d of $1.4 \mu M$ for the interaction of plasminogen with blood cells¹², then this level of Lp(a) would result in 16–24% occupancy of the binding sites on circulating blood cells with Lp(a), a 13–20% reduction in plasminogen binding and 3 – 4×10^6 Lp(a) molecules would be bound to each endothelial cell. This high level of vessel wall-associated Lp(a) could exert multiple effects on the endothelium, in addition to reducing the thrombolytic potential of the cell surface. Direct examination of the interaction of Lp(a) with cells will be the next essential step in evaluating these predictions. The present study suggests that the competition of Lp(a) and plasminogen for cellular binding sites may contribute to the thrombotic and atherosclerotic risks associated with elevated Lp(a) levels. □

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Lipoprotein(a) modulation of endothelial cell surface fibrinolysis and its potential role in atherosclerosis

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ENDOTHELIAL cells play a critical role in thromboregulation by virtue of a surface-connected fibrinolytic system. Cultured endothelial cells synthesize and secrete tissue-type plasminogen activator (t-PA)¹ which can bind to at least two discrete sites on the cell surface^{2–4}. These binding sites preserve the catalytic activity of t-PA and protect it from its physiological inhibitor (PAI-1)². N-terminal glutamic acid plasminogen (Glu-PLG), the main circulating fibrinolytic zymogen, also interacts specifically with the endothelial cell surface^{5,6}. Binding is associated with a 12-fold increase in catalytic efficiency of plasmin generation by t-PA⁵ which may reflect conversion of Glu-PLG to its plasmin-modified form, N-terminal lysine plasminogen (Lys-PLG)⁷. Lipoprotein(a) is an atherogenic^{8–11} lipoprotein particle which contains the plasminogen-like^{12,13} apolipoprotein(a) bound to low density lipoprotein. We report here that lipoprotein(a) interferes with endothelial cell fibrinolysis by inhibiting plasminogen binding and hence plasmin generation. In addition, we demonstrate lipoprotein(a) accumulation in atherosclerotic lesions. These findings may provide a link between impaired cell surface fibrinolysis and progressive atherosclerosis.

Lipoprotein(a) (Lp(a)) is a low-density lipoprotein (LDL)-like particle which contains, in addition to apolipoprotein B (apo(B)), the disulphide-linked apolipoprotein(a)^{14–17}. Apo(a) shares extensive structural homology with plasminogen, including multiple repeating domains similar to 'kringle' four, a single region similar to 'kringle' five and an inactive protease segment^{12,13}. Plasminogen and apo(a) are also genetically linked on chromosome 6 (ref. 18). We therefore investigated whether Lp(a) and apo(a) could modify the interaction between plasminogen and the endothelial cell. Lp(a) and apo(a) were tested for their ability to inhibit binding of ¹²⁵I-labelled Lys-PLG to cultured human umbilical vein endothelial cells (HUVEC) (Fig. 1). As we reported previously, unlabelled Lys-PLG blocked the specific interaction of ¹²⁵I-Lys-PLG in a dose-dependent fashion, giving a 50 per cent-inhibition dose (ID₅₀) corresponding to a 3.5-fold molar excess⁷ and 95% inhibition in the presence of a 50–100-fold molar excess of unlabelled Lys-PLG. When purified Lp(a) from one donor was tested, it gave an ID₅₀ equivalent to a 7-fold molar excess, indicating that its competitive activity closely resembled that of the unlabelled ligand.

Lp(a) from another donor gave an ID₅₀ equivalent to a 22-fold molar excess of competitor, and blocked 85% of plasminogen binding at a 50-fold excess. Apo(a) also inhibited binding of ¹²⁵I-Lys-PLG with an ID₅₀ corresponding to a 36-fold molar excess. By contrast with these results, neither LDL nor purified Lp(-), the reductively cleaved apo(a)-free particle, were effective in blocking binding of ¹²⁵I-Lys-PLG. These data suggest that the 'kringle'-containing apo(a) portion of the Lp(a) particle, when present in excess amounts, can significantly inhibit the binding of Lys-PLG to its main binding site.

In direct binding studies, ¹²⁵I-labelled apo(a) interacted with HUVEC in a dose-dependent fashion (Fig. 2). Consistent with an equilibrium existing between bound and unbound ligand, specific binding was found to be reversible (data not shown) by the infinite dilution method^{2,5,7}. Binding approached saturation for an input concentration of 400–800 nM and was half-maximal at ~300 nM. A Scatchard plot derived from the pro-

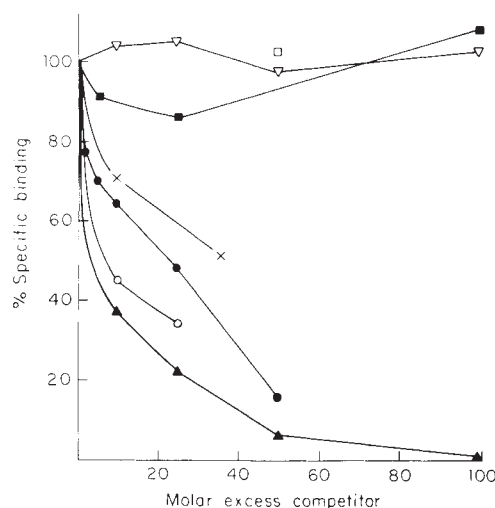


FIG. 1 Inhibition of ¹²⁵I-Lys-PLG binding to HUVEC by Lp(a) and apo(a). Confluent HUVEC monolayers were washed, treated with ϵ -aminocaproic acid, rewashed and incubated with ¹²⁵I-Lys-PLG (4.95 nM; specific activity 415,000 c.p.m. pmol⁻¹), for 30 min at 4 °C in the presence of various excess amounts of unlabelled Lys-PLG (▲), Lp(a) (●, ○), Apo(a) (×), LDL (■, □), or Lp(-) (▽) (see ref. 7). Specific binding was defined as that inhibited in the presence of 10 mM ϵ -aminocaproic acid⁵. Relative molecular mass of apo(a) was measured as described by Utermann *et al.*²². Each point represents the average of duplicate determinations.

METHODS. Plasma samples were freshly prepared from donors with high Lp(a) concentrations (> 40 mg dl⁻¹). Collections were made into 0.02% Na₃, 1 mM EDTA, 10 units ml⁻¹ kallikrein inhibitor, 0.001% phenylmethylsulphonyl fluoride, and 0.001% Synthomycin and immediately ultracentrifuged. The plasma was adjusted to 1.06 g ml⁻¹ and centrifuged at 150,000g for 24 h at 5 °C. The floating lipoproteins were removed, and the infranant adjusted with KBr to 1.12 g ml⁻¹ and recentrifuged. The 1.06–1.12 g ml⁻¹ fraction was dialysed against 20 mM Tris, 1 M NaCl, 0.02 Na₃, pH 7.4, and subjected to gel filtration on a Sephacryl S-400 HR column (2.6 × 90 cm) equilibrated with the same buffer. The fractions from the first visible UV-absorbing peak were monitored for Lp(a) and LDL by fast performance liquid chromatography (FPLC), mono Q chromatography and rocket electrophoresis. The purity of the preparation was assessed by SDS-PAGE (3–12%), by 3.75% native PAGE, and by western blotting using specific anti-apo(B) and anti-Lp(a) antibodies. Western blots and ELISAs confirmed that this fraction was free of apo(A-I) and apo(E). Apo(a) was purified from Lp(a) by reductive cleavage with dithiothreitol (DTT; 10 mM) in 0.15 M NaCl, 1 mM EDTA, 0.02% Na₃, 20 mM Tris, pH 7.4, for 3 h at 37 °C. The reduced Lp(a) was passed over a heparin-Sepharose column at low salt concentration (0.05 M NaCl). All of the applied apo(B) and lipid remained bound to the column. Two peaks of UV-absorbing material representing apo(a) and DTT were eluted. The apo(a) fraction was dialysed against column buffer without DTT, and either used fresh or lyophilized and stored at -20 °C. The fraction bound to the column eluted with high salt (0.5 M NaCl), and was found to contain apo(B) as the sole apoprotein. This fraction was designated Lp(-). LDL was prepared from donors with low Lp(a) levels from density fractions 1.025–1.055 g ml⁻¹.

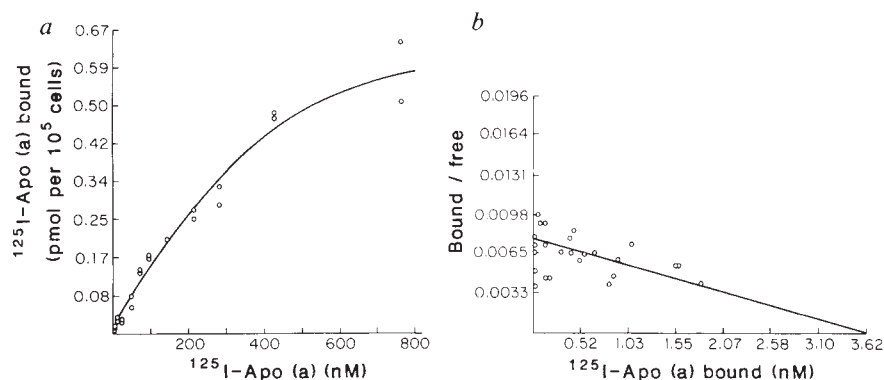


FIG. 2 Direct binding of apo(a) to HUVEC. *a*, Binding isotherm. HUVEC, prepared as described in Fig. 1, were incubated with various concentrations (0–760 nM) of ^{125}I -apo(a) for 30 min at 4 °C, labelled according to the lactoperoxidase method^{2,5,7} to specific activity 130,000 c.p.m. pmol^{-1} . Radioactivity, both bound and free, was determined as described^{2,5,7}. *b*, Scatchard plot. Data were analysed according to the program 'Ligand'¹⁹ and correspond to a single-site fit.

gram Ligand¹⁹ indicated the presence of a single binding site, with $K_d = 259$ nM and $B_{\text{max}} = 2.2 \times 10^6$ binding sites per cell. Interestingly, these parameters agree closely with binding constants determined for the chief binding sites for both Glu-PLG ($K_d = 310$ nM; $B_{\text{max}} = 1.4 \times 10^6$) (ref. 5) and Lys-PLG ($K_d = 120$ nM; $B_{\text{max}} = 390,000$) (ref. 7), which suggests that apo(a) is capable of interacting with HUVEC with an affinity and capacity comparable to that of plasminogen. It is known that plasma levels of Lp(a) vary widely ($< 0.01 \mu\text{M}$ – $1.0 \mu\text{M}$)^{20,21}.

Our results indicate that binding-site occupancy depends primarily on Lp(a) plasma levels: the binding isotherm indicates that although binding-site occupancy would be nearly complete

at apo(a) levels of $1 \mu\text{M}$, only a small fraction of binding sites would be occupied in individuals with low apo(a) levels.

We carried out functional studies to determine whether Lp(a) could inhibit the generation of plasmin by providing catalytic concentrations of tissue plasminogen activator at the cell surface. In a fluorogenic assay based on the synthetic plasmin substrate D-Val-Leu-Lys-aminofluoromethylcoumarin, Lys-PLG (6 nM), and t-PA (0.25 nM), Lp(a) (10 nM) had no effect on generation of plasmin in the fluid phase ($106.3 \pm 4.8\%$ of control). By sharp contrast, when an equivalent amount of surface-associated Lys-PLG was examined, generation of plasmin activity was reduced by $93.3 \pm 3.0\%$.

In addition to competing for plasminogen binding sites, Lp(a) could also directly inhibit t-PA activity or possibly block hydrolysis of the substrate by plasmin. To test the effect of Lp(a) on surface-associated t-PA, we incubated t-PA-bearing HUVEC with a fluorogenic t-PA substrate (2-Gly-Gly-Arg-aminofluoromethylcoumarin) in the presence of a 500-fold molar excess of either Lp(a) or LDL. Neither lipoprotein significantly inhibited t-PA function. The rate of substrate hydrolysis in the presence of LDL or Lp(a) was reduced by only 11.6% and 7.2% respectively. Similarly, when Lys-PLG-bearing endothelial cells were exposed to catalytic concentrations of t-PA pre-incubated with excess amounts of fluid phase Lp(a), no effect on plasmin generation was observed, as judged by a lack of hydrolysis of the D-Val-Leu-Lys-aminofluoromethylcoumarin substrate. Furthermore, Lp(a) had no effect on the activity of fluid phase plasmin. It seems therefore that modulation of plasmin generation at the endothelial cell surface by Lp(a) reflects a specific effect confined to competition with plasminogen for its cell-surface binding site.

In previous immunohistochemical studies, we demonstrated the presence of Lys-PLG on the endothelial surface of vessels from a wide variety of tissues⁷. For these studies, we used a monoclonal antibody directed specifically against Lys-PLG which was unreactive against either Glu-PLG or Lp(a). Given the apparent proclivity of Lp(a) for the endothelial cell surface and its ability to inhibit cell surface plasmin generation, we investigated whether this lipoprotein could also be identified in vessels from normal and abnormal human tissue (Fig. 3). For these immunohistochemical studies, we used a monospecific polyclonal antibody directed against human Lp(a), and immunoadsorbed to extinction with human plasminogen and apo(B-100), as verified by western blot analyses. These studies revealed a striking accumulation of immunoreactive material associated with the endothelium and intimal subendothelium of atherosclerotic coronary arteries derived from autopsy specimens (Fig. 3a). These vessels showed only minimal amounts of stainable plasminogen at the vessel surface. Tissue from a saphenous vein aortico-coronary by-pass graft from the same individual showed even larger amounts of immunoperoxidase-positive material within the vessel wall (Fig. 3c). No staining was observed in either vessel when the primary antibody was pre-adsorbed with Lp(a) (Fig. 3b, d). Staining persisted when the antibody was adsorbed with Lp(-) or LDL, confirming that

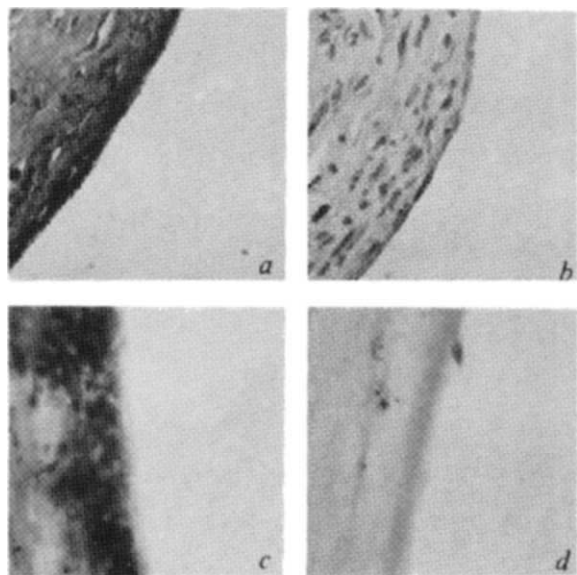


FIG. 3 Immunohistochemical identification of Lp(a) in atherosclerotic vessels obtained at autopsy. The patient's serum Lp(a) level was unknown. Serial sections of a formalin-fixed paraffin-embedded atherosclerotic coronary artery (*a, b*) and atherosclerotic saphenous vein aortico-coronary by-pass from the same individual; (*c, d*) were stained with horse anti-Lp(a) adsorbed with plasminogen and apo(B) (*a, c*), or horse anti-Lp(a) adsorbed with plasminogen, apo(B), and Lp(a) (*b, d*). Magnification 125 $\times$.

METHODS. Monospecific anti-Lp(a) was raised in horses (by G. M. Kostner, Graz, Austria) and adsorbed to extinction by western blot with both human plasminogen and human apo(B) on nitrocellulose strips. Cross-reactivity with plasminogen, LDL, and fibrinogen were all negative. Formalin-fixed, paraffin-embedded sections were dewaxed and pronase-treated, and endogenous peroxidase activity was blocked by treatment with hydrogen peroxide (3%, 30 min). The slides were preincubated with 10% normal horse serum in buffer for 1 h and then incubated for 18 h at 4 °C with primary antibody (horse anti-Lp(a), 1/4,000 dilution). After a brief blocking step with normal horse serum, the slides were exposed to biotinylated goat-anti horse IgG (1/250 dilution) for 30 min at 21 °C, and incubated with avidin-biotin peroxidase complex. Peroxidase deposition was visualized with 3,3'-diaminobenzidine tetrahydrochloride, and sections were counterstained with haematoxylin.

the immunoreactive material was apo(a)-related. Medium-sized arteries with minimal atherosclerotic disease from the liver and kidney of the same patient displayed no Lp(a) staining. When normal coronary vessels from another patient were studied in parallel, there was no detectable staining for Lp(a). These findings suggest that, in some individuals, the atherosclerotic state may be associated with accumulation of Lp(a) within the vessel wall.

Recent studies have suggested that the endothelial cell possesses a cell-surface fibrinolytic system, the regulation of which has been unclear. This system includes binding sites for plasminogen and t-PA. The juxtaposition of these two substances would result in the continuous generation of cell surface plasmin protected from its inhibitor (α_2 -plasmin inhibitor) by the occupancy of plasmin lysine-binding sites at the cell surface.

The surface-localized conversion of Glu- to Lys-PLG⁷, which is sensitive to diisopropyl fluorophosphate, is consistent with the presence of a plasmin-like serine protease on the endothelial cell surface. Whereas physiological levels of Lp(a) would serve to control the unopposed generation of plasmin at the cell surface, elevated levels of Lp(a) would suppress the cell's 'sentinel' fibrinolytic mechanism, leading to a prothrombotic tendency. Thus, the association of large amounts of immunodetectable Lp(a) on and within the lesions of diseased coronary vessels may provide a link between impaired cellular fibrinolysis and progressive atherosclerosis.

Note added in proof: Since submission of this manuscript, M. Gonzalez-Gronow *et al.*²³ have reported evidence of competitive binding of plasminogen and lipoprotein(a) at a common site on U937 cells. □

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No linkage of chromosome 5q11-q13 markers to schizophrenia in Scottish families

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RECENT work suggests that an autosomal dominant gene for schizophrenia may be located on the 5q11-q13 region of chromosome 5 (refs 1 and 2): a report of schizophrenia associated with trisomy 5q11-q13 in two members of a family of Chinese origin¹ prompted the discovery of linkage with markers p105-599Ha and p105-153Ra in five Icelandic and two English schizophrenic families². The strongest linkage was observed when the phenotype was broadly defined to include minor psychiatric diagnoses not traditionally considered part of the schizophrenia spectrum. By contrast, no evidence was found of linkage in a single multiplex Swedish schizophrenic pedigree³. To determine whether these conflicting results arise from genetic and/or uncertainties in defining the schizophrenic phenotype, we examined fifteen Scottish schizophrenic families with restriction fragment length polymorphisms that span this region. We found no evidence for linkage, regardless of how broadly or narrowly the schizophrenic phenotype is defined, and conclude that a susceptibility locus, whose presence awaits confirmation, on the proximal portion of the long arm of chromosome 5 can be responsible for only a minority of cases of familial schizophrenia.

In our analysis, we used approximately the same definitions of the schizophrenia phenotype as used by Sherrington *et al.* Thus, fifteen families were serially entered into the study after

being considered suitable for restriction fragment length polymorphism (RFLP) studies by criteria which included: two index cases who met Research Diagnostic Criteria (RDC)⁴ and criteria

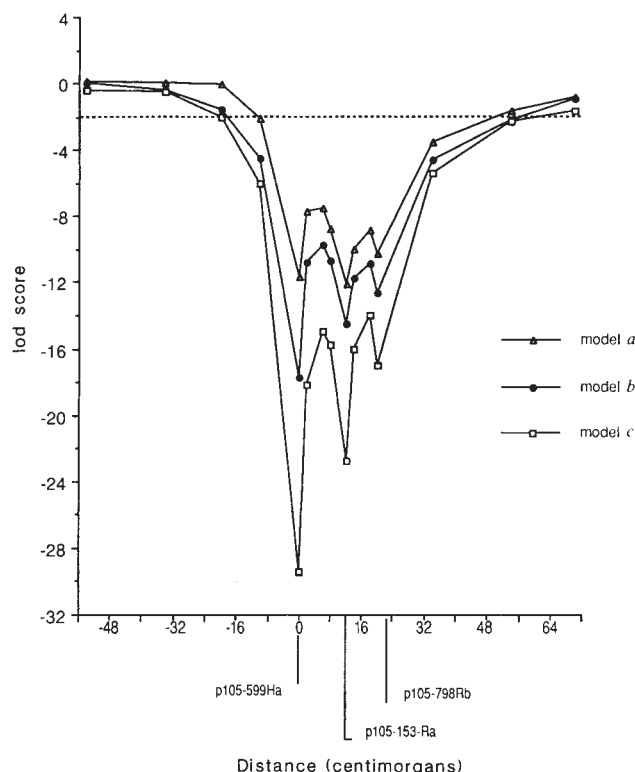
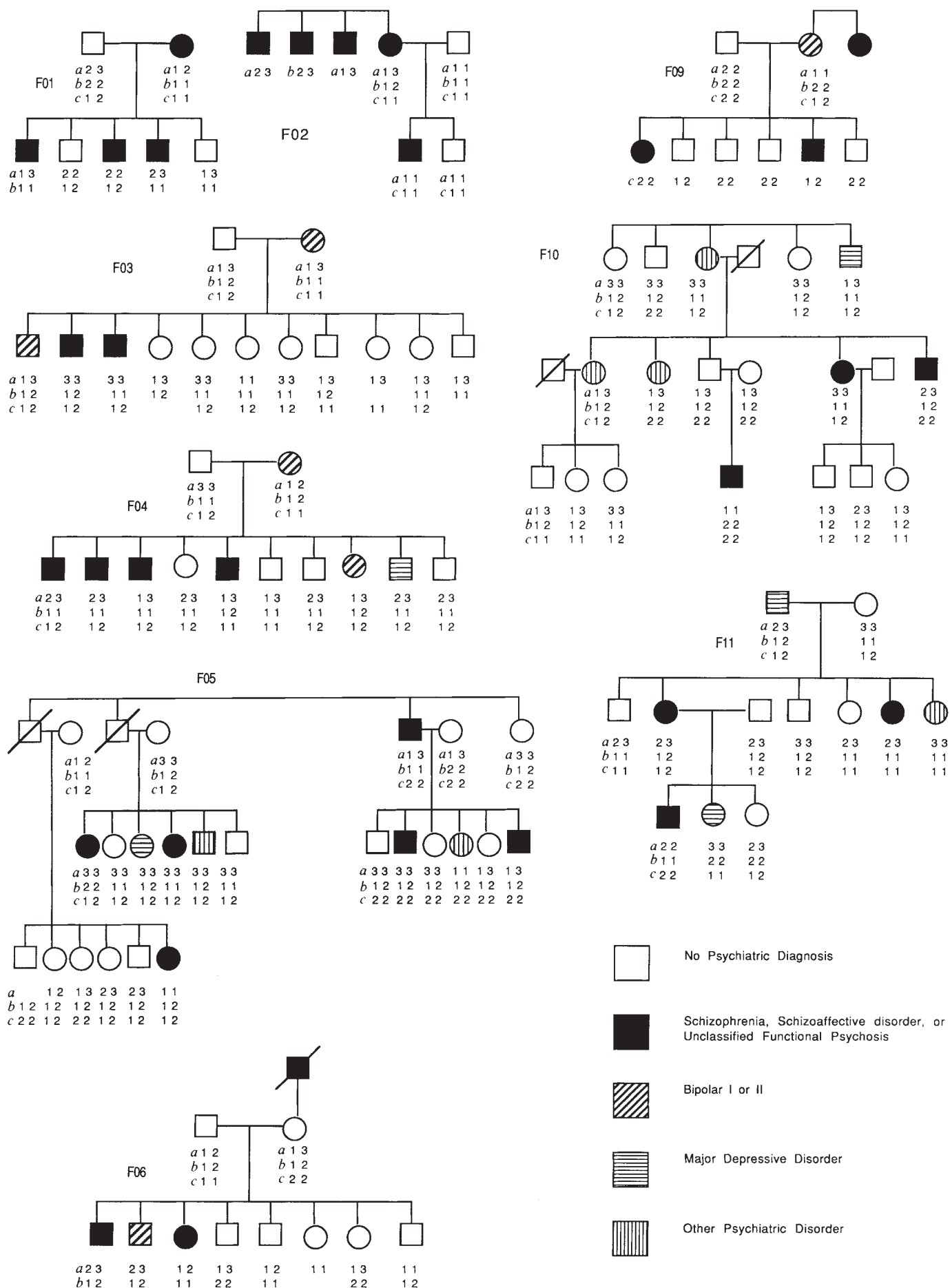


FIG. 1 Clinical psychiatric diagnoses by RDC⁴ and RFLP-typing data on individual members of 15 pedigrees. Minor modifications have been made to preserve confidentiality. All families lived in Scotland, and were mostly of Scottish but also of English, Irish and Italian origin. Alleles of probes are numbered in order of decreasing fragment size, as described in the legend to Table 1.



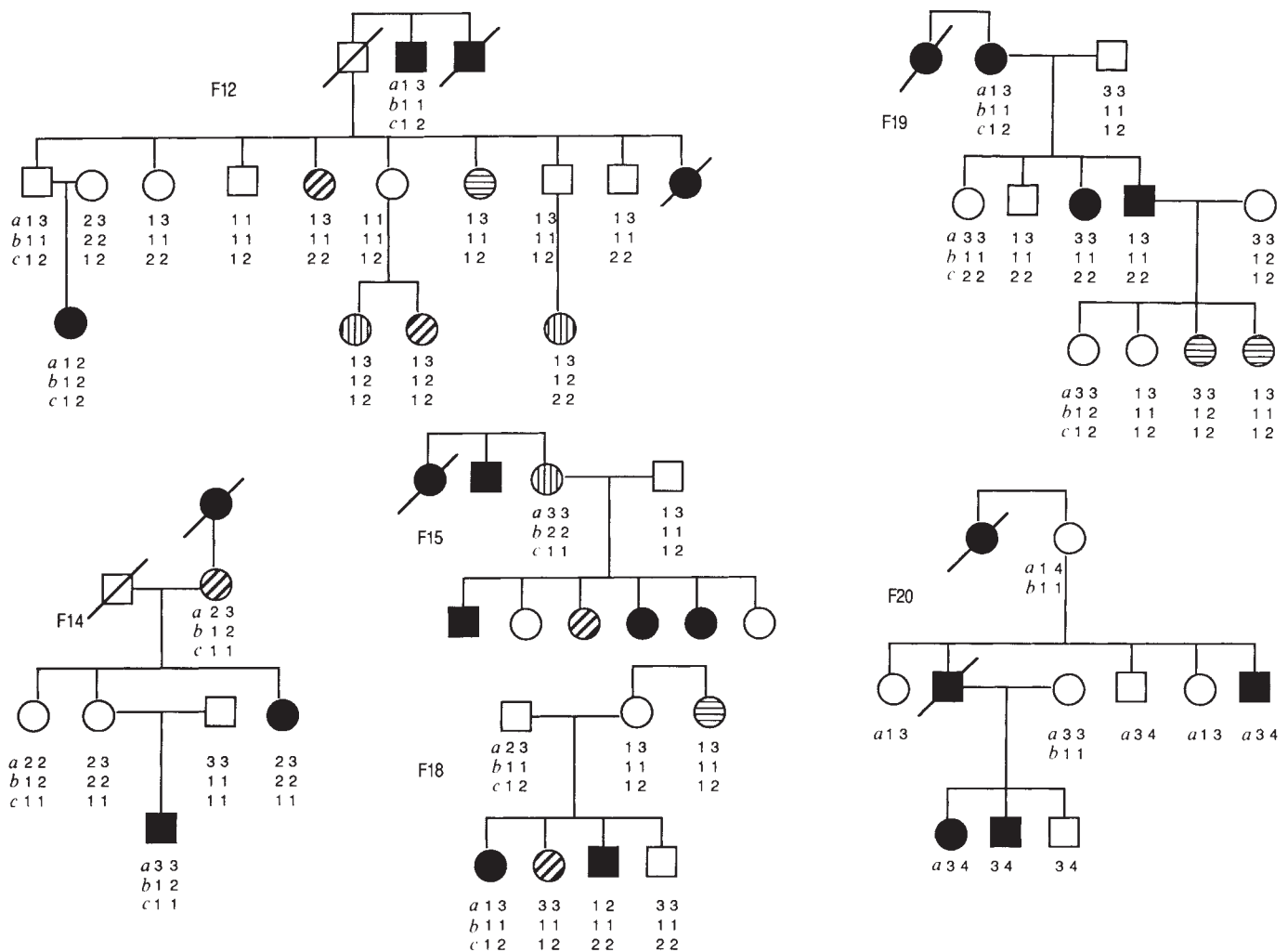


FIG 2 Four-point analysis from the LINKMAP programme of LINKAGE¹⁴. The schizophrenia locus moves across a fixed map of p105-599Ha, p105-153Ra and p105-798Rb. p105-599Ha was set at 0, p105-153Ra at 0.12 M and p105-798Rb at 0.2 M (male distances). Female/male ratio of recombination was 1.5. Model *a* included as cases schizophrenia, bipolar illness, schizoaffective disorder, and unspecified functional psychosis; model *b*, as

in *a*, with major depressive disorder also included; model *c*, all cases with an RDC diagnosis. Four-point analysis was not performed on model *d* (schizophrenia, schizoaffective disorder and unspecified functional psychosis), but the two-point lod score on p105-599Ha, the most informative probe, was -3.1 at 5% recombination and -1.6 at 10% recombination.

defined by the American Psychiatric Associations Diagnostic and Statistical Manual of Mental Disorders (DSM III R)⁵ for definite schizophrenia; major mental illness in two or, for preference, three generations; large sibship size; and no evidence, as far as could be reasonably ascertained, of assortative mating. Families were identified from hospital case registers or referred by psychiatric colleagues, mainly from the south of Scotland. Initial contact with families was made through psychiatrists looking after the index cases. Family members were fully informed about the nature of the study and those taking part agreed to a structured interview using the Schizophrenia and Affective Disorders Schedule—lifetime version (SADS-L)⁶, and recordings of smooth pursuit eye movements and auditory-event-related potentials, accounts of which formed part of a study of psychophysiological traits in these families (D.B. *et al.*, manuscript in preparation). The subjects also agreed to venepuncture, performed for molecular genetic studies. Paternity was confirmed by red-cell typing and histocompatibility antigens.

DNA was prepared from 166 members, all of whom were interviewed by one of the authors (D.St.C., D.B. or W.M.). A consensus diagnosis on each family member was made by the three psychiatrists involved on the basis of the clinical interview, hospital case record review and, where appropriate, by consultation with clinical colleagues in charge of cases. Major mental

disorders present among those used for RFLP-typing were, by RDC, definite schizophrenia ($n=44$), schizoaffective disorder ($n=2$), bipolar I ($n=5$), bipolar II ($n=5$), unspecified functional psychosis ($n=2$), and major depressive disorder ($n=8$). An additional nine subjects had other psychiatric disorders (five with minor depressive disorder, one with schizotypal personality disorder, and one each with generalized anxiety disorder, chronic alcoholism, and obsessive-compulsive disorder). In eight families, major mental illness was identified in three generations, in seven families in two only.

We looked for linkage using three markers mapping to the proximal portion of the long arm of chromosome 5, p105-599Ha, p105-153Ra, and p105-798Rb. These markers span a distance of ~25 centimorgans and cover the trisomic region associated with schizophrenia^{3,7}. Because of uncertainty about which psychiatric diagnoses should be included as part of the schizophrenia spectrum⁸⁻¹³, we analysed our data using four separate models (*a*, *b*, *c* and *d*). In *a*, affected cases were restricted to schizophrenia, schizoaffective disorder, bipolar disorder, and unspecified functional psychosis (penetrance, 66%), whereas in *b*, major depressive disorder was also included (penetrance, 77%). In *c*, all family members with RDC psychiatric diagnoses, including schizotypal personality disorder, were considered as cases (penetrance, 94%). In *d*, the data on p105-599Ha, the

most informative probe, were also analysed, restricting affected cases to schizophrenia, schizoaffective disorder and unspecified functional psychosis (penetrance, 44%). We assumed an autosomal dominant mode of inheritance with a gene frequency for schizophrenia of 0.0085. The penetrance values were calculated from our own complete data set of 197 subjects in 19 families. Figure 1 gives RFLP-typing data on the individual family members and Table 1 shows the individual lod scores for each marker for informative families using the assumptions of model *b*. Figure 2 shows the four-point analysis for models *a*, *b* and *c*.

Our data show no evidence of linkage of a schizophrenia susceptibility allele to the proximal portion of the long arm of

chromosome 5. It is unlikely that our findings differ from those of Sherrington *et al.* as a result of the method of analysis. We used almost identical models for the schizophrenia phenotype and similar inheritance parameters. Nor was the presence in some families of cases of bipolar affective disorder a likely explanation. In six families (F1, F2, F5, F11, F19 and F20 in Table 1) there were no cases of bipolar illness, yet the lod score of these families, analysed separately for p105-599Ha, was -2 or less at 10% recombination, using models *a*, *b* and *c*. It seems more probable that a susceptibility allele in this region, whose presence we have failed to confirm, could be responsible for only a minority of cases of familial schizophrenia. □

TABLE 1 Two-point lod scores at specified recombination fractions for linkage between schizophrenia and chromosome 5 markers

Family	Probe	Recombination fraction (θ)						
		0.001	0.05	0.1	0.15	0.2	0.3	0.4
F1	a	-2.10	-0.80	-0.56	-0.38	-0.26	-0.10	-0.02
	b	—	—	—	—	—	—	—
	c	—	—	—	—	—	—	—
F2	a	-1.10	-0.69	-0.48	-0.34	-0.24	-0.10	-0.02
	b	—	—	—	—	—	—	—
	c	—	—	—	—	—	—	—
F3	a	-0.34	-0.27	-0.20	-0.15	-0.11	-0.05	-0.01
	b	—	—	—	—	—	—	—
	c	—	—	—	—	—	—	—
F4	a	-1.90	-1.70	-1.30	-0.86	-0.57	-0.23	-0.05
	b	-1.90	-1.80	-1.40	-1.00	-0.68	-0.27	-0.06
	c	—	—	—	—	—	—	—
F5	a	-1.50	-1.20	-0.85	-0.86	-0.41	-0.17	-0.04
	b	-2.20	-1.00	-0.60	-0.38	-0.25	-0.09	-0.02
	c	-0.08	-0.06	-0.04	-0.03	-0.02	-0.01	-0.002
F6	a	-2.30	-0.87	-0.55	-0.36	-0.24	-0.09	-0.02
	b	-0.004	+0.01	+0.02	+0.02	+0.02	+0.01	+0.03
	c	—	—	—	—	—	—	—
F9	a	—	—	—	—	—	—	—
	b	—	—	—	—	—	—	—
	c	-1.05	-0.69	-0.47	-0.33	-0.23	-0.10	-0.02
F10	a	—	—	—	—	—	—	—
	b	-1.60	-0.41	-0.20	-0.11	-0.07	-0.05	-0.05
	c	-0.21	-0.07	+0.02	+0.07	+0.09	+0.07	+0.03
F11	a	-1.05	-0.69	-0.47	-0.33	-0.23	-0.10	-0.02
	b	-2.00	-0.79	-0.47	-0.30	-0.19	-0.07	-0.01
	c	-3.00	-1.80	-1.20	-0.84	-0.60	-0.28	-0.09
F12	a	-1.83	-0.26	-0.06	+0.01	+0.03	+0.02	+0.008
	b	—	—	—	—	—	—	—
	c	+0.07	+0.06	+0.05	+0.03	+0.02	+0.01	+0.003
F14	a	—	—	—	—	—	—	—
	b	+0.50	+0.43	+0.36	+0.29	+0.23	+0.12	+0.04
	c	+0.40	+0.35	+0.30	+0.25	+0.19	+0.09	+0.02
F18	a	—	—	—	—	—	—	—
	b	-1.50	-0.41	-0.20	-0.10	-0.05	-0.01	-0.001
	c	—	—	—	—	—	—	—
F19	a	-0.32	-0.22	-0.16	-0.11	-0.08	-0.03	-0.007
	b	-3.30	-1.70	-1.10	-0.76	-0.52	-0.21	-0.05
	c	—	—	—	—	—	—	—
F20	a	-0.55	-0.45	-0.35	-0.26	-0.19	-0.08	-0.02
	b	+0.47	+0.48	+0.47	+0.44	+0.39	+0.27	+0.13
	c	—	—	—	—	—	—	—
Totals	a	—	—	—	—	—	—	—
	b	-18.1	-7.52	-4.88	-3.38	-1.97	-0.64	-0.05
	c	-8.23	-4.59	-3.03	-2.03	-1.37	-0.58	-0.17
		-4.04	-1.75	-1.06	-0.65	-0.40	-0.13	-0.02

Probes: a, p105-599Ha; b, p105-153Ra; and c, p105-798R6. Analysis was performed using MLINK¹⁴. The gene frequency for schizophrenia was 0.0085. Model *b* (see text) was used to define the schizophrenia phenotype with a penetrance of 77%. DNA was prepared from peripheral blood lymphocytes, digested with restriction enzymes and prepared for RFLP typing by the established procedure of gel electrophoresis, transfer to nylon membrane (Hybond-N) and either nick-translation or oligonucleotide-labelling. The p105-599Ha probe contained a repeat sequence and was therefore stripped by pre-hybridization with a large excess of denatured DNA. Probe p105-599Ha (D5S76) is defined by a *Taq* I polymorphism giving a four-allele system with 17, 14, 10, and 9-kilobase (kb) fragments at frequencies of 0.32, 0.16, 0.50, and 0.02, respectively. The last, rare, and previously unreported allele was found by ourselves and by Sherrington *et al.* (personal communication) at the above frequency. p105-153Ra (D5S39) has an *Xba* I polymorphism with 6.3- and 5.2-kb fragments at frequencies of 0.71 and 0.29 respectively. p105-798Rb (D5S78) has an *Msp* I polymorphism with 2.3- and 1.8-kb fragments of frequencies 0.57 and 0.43. The three probes map to 5q11-13 and have been described previously³. One family was uninformative for all three probes and is omitted from the table. Blanks are left in the table where probes were uninformative.

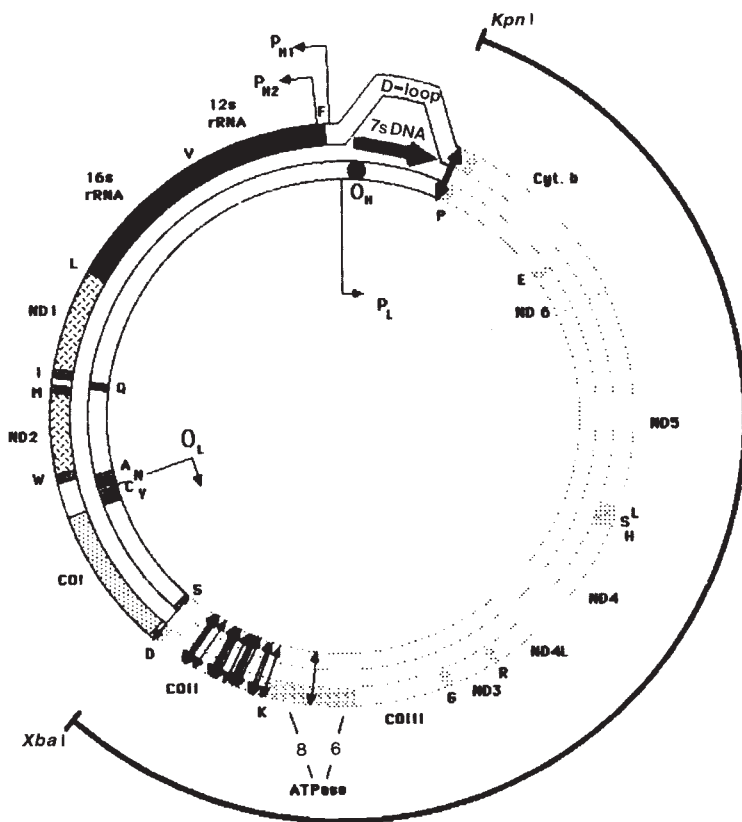


FIG. 3 Map of human mtDNA showing the localization and extension of the multiple deletions found in the patients. Outer and inner circles represent the heavy (H) and the light (L)-strand, respectively. Deletions were deduced by both restriction mapping analysis², and sequencing of the breakpoint regions. The restriction endonucleases used were *PvuII*, *XhoI*, *BamHI*, *PstI*, *EcoRI*, *HindIII*, *KpnI*, *XbaI* and *StuI*. *XbaI* at bp 7,440 and *KpnI* at bp 16,133, the conserved sites nearest to each side of the breakpoints, are indicated. They represent the restriction-map limits of the region containing all the deletions (solid circular line). The hatched area represents the maximal extension of the deletions, deduced by sequence analysis. Thick and thin double arrows indicate 3' and 5' positions of the breakpoints, respectively⁵. P_{H1} , major heavy strand promoter; P_{H2} , minor heavy strand promoter; O_H , origin of heavy (H)-strand replication; O_L , origin of light (L)-strand replication; 12S rRNA and 16S rRNA, mitochondrial ribosomal RNAs; ND 1-6, NADH dehydrogenase subunits; CO I-III, cytochrome c oxidase subunits. Single capital letters indicate amino-acid-specific tRNA genes.

purified from human liver². All the DNA samples showed a hybridization band of ~16.5 kilobases (kb), corresponding to 'wild-type' mtDNA. Several additional bands in the range 8.0–12.5 kb, however, were found exclusively in the four propositi, (Fig. 2a), and were interpreted as corresponding to as many linearized mtDNA molecules. Additional endonucleases, which cleave human mtDNA at two or more sites (see legend of Fig. 2b), were used for further restriction mapping. The results of this mapping were compatible with the presence of multiple deletions that, in all four patients, affected the same mtDNA region, found within a restriction site for *XbaI* (bp 7,440; ref. 5), and a restriction site for *KpnI* (bp 16,133) (Fig. 3).

In a second set of experiments, the same *PvuII*-digested DNAs were probed with a radiolabelled fragment encompassing the mtDNA region from bp 14,955 (restriction site for *XhoI*) to bp 16,052 (restriction site for *KpnI*). Because the mutant mtDNAs were not detected by the probe (data not shown), the hybridization pattern showed only the ~16.5-kb band corresponding to normal mtDNA. These results indicate that all the 'breakpoints' of the deletions are localized between the conserved *KpnI* site at bp 16,133 and the lost *KpnI* site at bp 16,052.

Further analysis was carried out after polymerase-chain-reaction (PCR) amplification⁶ of the segments containing the breakpoints. Mitochondrial genomic DNA from each patient was primed by two oligonucleotides, which encompassed the conserved restriction sites for *XbaI* and *KpnI*, (Fig. 3). Numerous fragments, of lengths 80–2,000 bp, were generated by PCR (Fig. 2b), digested with *KpnI* and *XbaI*, and cloned into suitable plasmids. Among the several 'clonal species' identified by size in each patient, we sequenced eight clones from patient III-4, two from patient III-6, two from patient III-7, and seven from patient II-6 (Fig. 4a). All sequences had a common feature: the 3' termini of the breakpoints (mtDNA L-strand orientation) were consistently localized in a 12-nucleotide cluster, from bp 16,068 to bp 16,079 (Fig. 4a, b), in the D-loop region of mtDNA. The 5' termini, on the other hand, extended across a region of

~1,000 bp, including the genes coding for subunit II of cytochrome c oxidase, the tRNAs for Ser and Asp and subunits VIII and VI of the mitochondrial ATPase⁵. The reason for the latter variability is unclear. However, in each of the mutant mtDNAs analysed by sequencing, small 'boxes' of nucleotide homology are identifiable at each edge of the deletion (Fig. 4a). Thus, deletions seem to be produced by homologous recombination at specific sites, indicating that the causative mechanism is specific.

All the deletions started at the 5' end of the mtDNA D-loop template (L-strand configuration). The mtDNA D-loop is a region where protein-DNA interactions are thought to occur⁷, directing both mtDNA replication⁷ and transcription⁸. All the breakpoints of the deletions were localized at a few nucleotides downstream from the template region coding for the 3' terminus of the D-loop heavy(H)-strand⁹, near to sequences which, because of their high conservation, or their potential secondary structure, are of possible functional significance (Fig. 4b). In particular, the template sequence at bp 16,157–16,172 (ref. 5), has been implicated as a putative template stop signal, involved in the termination of D-loop H-strand synthesis in human⁹, mouse⁹, pig¹⁰ and bovine^{11,12} mtDNAs. The sequence at bp 16,087–16,101, is highly conserved in human⁵, mouse¹³, pig¹⁰ and rat¹⁴ mtDNAs, and is capable of forming a hairpin secondary structure. A second potential hairpin structure, present only in the human template, is localized at bp 16,043–16,061, just downstream from the breakpoints. These sequences seem to be candidates for specific interaction(s) with the mtDNA polymerase, or any associated replication factors, in template-sequence directed events leading to either the stop of D-loop strand synthesis, or the start of H-strand elongation, or both^{7,9,10}. The fact that all the breakpoints were consistently found to be associated with these structures, points to the existence of an etiologic correlation between the mechanism generating the lesions and the functions of the D-loop.

The genetic analysis indicates unequivocally that in this family

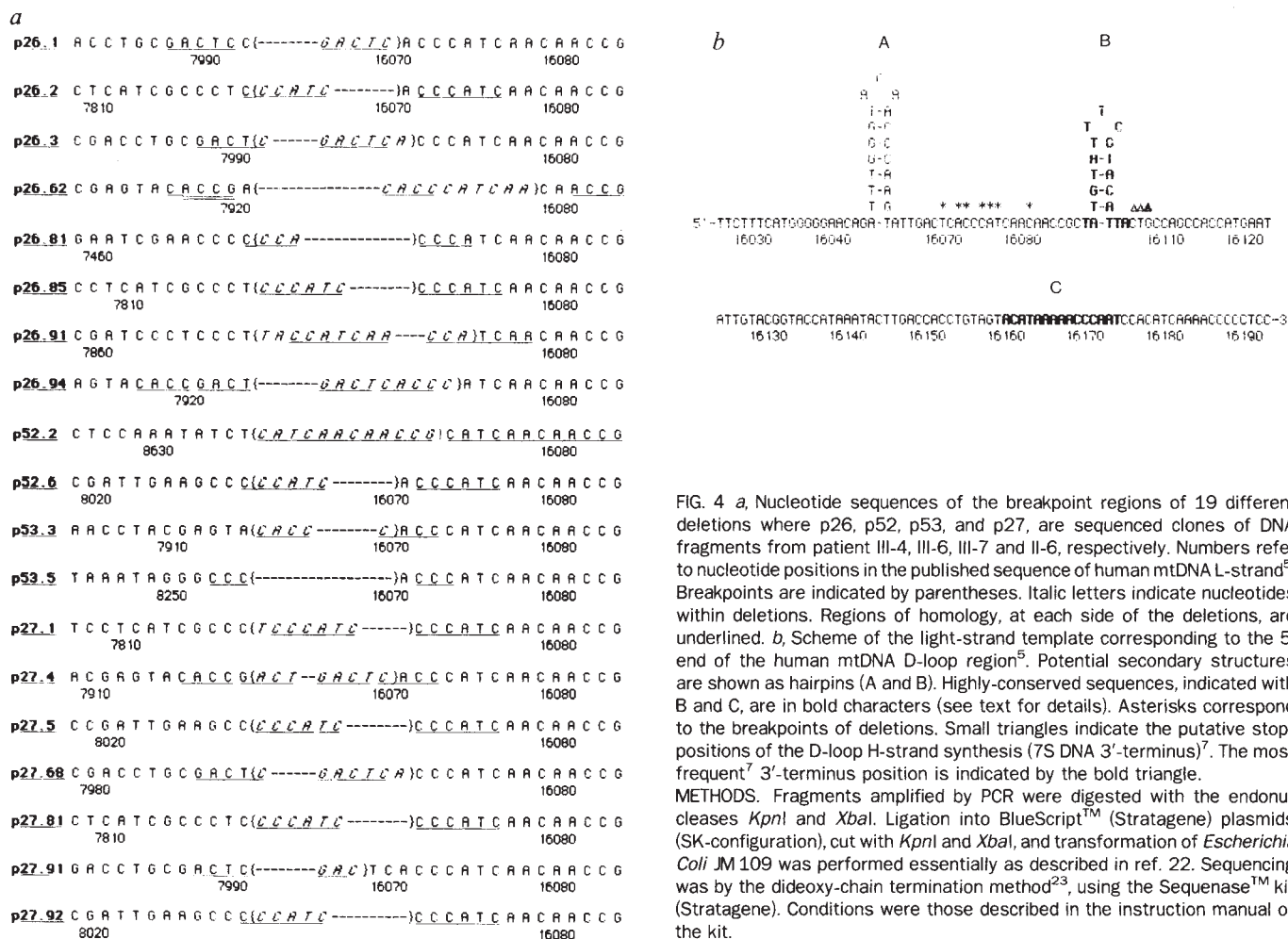


FIG. 4 *a*, Nucleotide sequences of the breakpoint regions of 19 different deletions where p26, p52, p53, and p27, are sequenced clones of DNA fragments from patient III-4, III-6, III-7 and II-6, respectively. Numbers refer to nucleotide positions in the published sequence of human mtDNA L-strand⁵. Breakpoints are indicated by parentheses. Italic letters indicate nucleotides within deletions. Regions of homology, at each side of the deletions, are underlined. *b*, Scheme of the light-strand template corresponding to the 5' end of the human mtDNA D-loop region⁵. Potential secondary structures are shown as hairpins (A and B). Highly-conserved sequences, indicated with B and C, are in bold characters (see text for details). Asterisks correspond to the breakpoints of deletions. Small triangles indicate the putative stop-positions of the D-loop H-strand synthesis (7S DNA 3'-terminus)⁷. The most frequent 3'-terminus position is indicated by the bold triangle.

METHODS. Fragments amplified by PCR were digested with the endonucleases *KpnI* and *XbaI*. Ligation into BlueScriptTM (Stratagene) plasmids (SK-configuration), cut with *KpnI* and *XbaI*, and transformation of *Escherichia coli* JM 109 was performed essentially as described in ref. 22. Sequencing was by the dideoxy-chain termination method²³, using the SequenaseTM kit (Stratagene). Conditions were those described in the instruction manual of the kit.

both the clinical picture and the mtDNA deletions are produced by an autosomal dominant mechanism. Maternal inheritance is ruled out by the recurrent transmission of the clinical and the molecular traits to patrilinear descendants (Fig. 1). Furthermore, we never found the same breakpoint in PCR-clones of different individuals (Fig. 4*a*), suggesting that the heteroplasmic mtDNA populations are not transmitted *per se*, but are rather generated *de novo* by a common mechanism.

This is the first example of a mendelian trait associated with lesions of the human mitochondrial genome, which acts as the target of a mutant nuclear-coded, trans-acting factor. The deleterious effects of this factor are expected to be cumulative in time, a possible explanation of both the late onset and the progressive course of the disorder. Because of the strategic position at which the molecular lesions occur, the role of this factor is likely to influence general 'housekeeping' functions of mtDNA gene expression.

Knowledge of the proteins involved in gene replication and/or transcription of mammalian mitochondrial genes is far from complete. In addition to mitochondrial primase¹⁵, mitochondrial γ -DNA-polymerase¹⁶, and mitochondrial topoisomerases I¹⁷ and II¹⁸, a protein of relative molecular mass 16,000, strongly bound to the D-loop region, has been identified in the rat¹⁹. Its proposed role is to prevent parental branch migration, by stabilizing the nascent H strand at the template¹⁹. This protein is analogous to a single-stranded DNA-binding protein (SSB), isolated from *Xenopus laevis* mitochondria²⁰, which, in turn, shows many similarities²⁰ with the prokaryotic SSBs, which direct replication by holding the template in a suitable, unfolded form. Furthermore, a single-stranded, DNA-specific endonu-

lease from mouse cell mitochondria has been recently identified²¹; its function is similar to fungal mitochondrial enzymes, whose absence correlates with defective DNA repair and recombination²¹. Our study indicates the possible implications for human pathology of these and other, as yet unknown, factors, also involved in the 'cross-talk' between the nuclear and the mitochondrial genomes. □

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CD2-mediated adhesion facilitates T lymphocyte antigen recognition function

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THE CD2 T lymphocyte-surface glycoprotein serves to mediate adhesion between T lymphocytes and their cognate cellular partners which express the specific ligand LFA-3 (refs 1–3). In addition, CD2 by itself or in conjunction with T-cell receptor stimulation, transduces signals resulting in T-lymphocyte activation^{4–8}. One or both of these functions seems to be physiologically important, given that certain anti-CD2 monoclonal antibodies block T-cell activation^{9,10} and that antigen-responsive memory T cells express a high level of CD2 relative to virgin T cells, which are largely antigen-unresponsive¹¹. Nevertheless, the contribution of the individual CD2 functions in T-cell responses has not been independently examined. To this end, human CD2 complementary DNAs encoding an intact LFA-3-binding adhesion domain, but lacking a functional cytoplasmic signal transduction element (CD2_{trans}[−]), were introduced into an ovalbumin-specific, I-A^d restricted murine T-cell hybridoma. The antigen-specific response of T hybridoma cells expressing human CD2_{trans}[−] protein was enhanced up to 400% when the human LFA-3 ligand was introduced into the I-A^d expressing murine antigen-presenting cells. In contrast, no augmentation was observed if human LFA-3 was absent or expressed on a third-party cell lacking the I-A^d restriction element. These results directly demonstrate the functional significance of adhesion events mediated between CD2 on the antigen-responsive T lymphocyte and LFA-3 on the presenting cell in optimizing antigen-specific T-cell activation.

Gene transfer studies in T cells using vectors containing wild type or truncated CD2 cDNAs have identified the importance of cytoplasmic residues for activation^{8,12}, particularly between positions 253 and 287 of human CD2, which includes the histidine and proline-rich region (amino acids 253–278) (Table 1; ref. 13). The present study was conducted to determine whether the cytoplasmic region of CD2 influences the ability of the extracellular segment to interact with LFA-3, and whether there is a significant contribution of CD2-mediated adhesion *per se* in the functional recognition of antigen by the T-cell receptor. To examine the interaction of truncated CD2 mutants with LFA-3, a rosetting assay with sheep red blood cells (SRBC) expressing the sheep LFA-3 homologue³ was used. As shown in Fig. 1a and as expected, the parental murine T-hybridoma cell line, 3DO54.8, which lacks human CD2, does not form rosettes with SRBC. By contrast, 3DO54.8 cells transfected with either the full-length human CD2 cDNA (CD2FL) (not shown) or truncated human CD2 cDNAs which retain 98 or 43 amino acids of the 117-residue cytoplasmic domain (CD2ΔC98 and CD2ΔC43), form rosettes which are readily detectable as aggregates of SRBC around an individual T lymphocyte (Table 1). This is the case for both the truncated form of CD2 capable of mediating signal transduction after external segment perturbation (CD2ΔC98), as well as for the truncation (CD2ΔC43) which has lost the capacity to transduce activation signals.

To test whether truncations of the cytoplasmic region altered the affinity of CD2 for LFA-3, competition analysis was performed by determining the concentration of a monomeric, water-soluble recombinant CD2 extracellular segment, termed T11_{ex2} (amino acids 1–182) (ref. 14), required to half-maximally inhibit

SRBC rosetting. Figure 1b shows that similar competition curves are obtained for CD2FL and CD2ΔC43 with 50% inhibitory concentration of ~0.8 and 0.9 μM, respectively. These data indicate that a truncated CD2 molecule which is unable to transduce activation signals binds to LFA-3 with affinity ($K_d \sim 0.9 \mu\text{M}$) comparable to the intact, full-length CD2 structure ($K_d \sim 0.8 \mu\text{M}$). The latter measurement is in good agreement with K_d determined for the T11_{ex2}-LFA-3 interaction by Scatchard analysis of saturable binding data¹⁴. This result could not be predicted from the precedent with another adhesion molecule, E cadherin; structural alterations in the E cadherin cytoplasmic domain dramatically reduce ligand binding capacity¹⁵.

Having dissociated CD2-mediated adhesion from activation, it was next possible to evaluate independently the role of adhesion in an immune response. For this purpose, we tested the ability of CD2_{trans}[−] and CD2_{trans}⁺ transfectants of the 3DO54.8 T-cell line to produce IL-2 upon stimulation with the antigen-pulsed I-A^d B-cell line A20, or an A20 transfectant P24A20 (schematically depicted in Fig. 2b) expressing the human cDNA encoding the phosphatidyl inositol-linked form of LFA-3 (Fig. 2a).

As shown in Fig. 2c (left panel), the parental cell line 3DO54.8 produces equivalent amounts of IL-2 upon ova stimulation, regardless of whether A20 or P24A20 is used as the antigen-presenting cell. This implies that there is no substantial difference

TABLE 1 Functional characteristics of murine T-T hybridomas expressing wild-type or variant forms of human CD2

Cell line	CD2 functional status	CD2 surface expression, molecules per cell	Amino acid residues in the cytoplasmic domain	Stimulation with anti-T11 ₂ + anti-T11 ₃ [Ca ²⁺] ²⁺ rise	IL-2 secretion
3DO54.8	NA	0	0	—	—
CD2FL	trans ⁺	5,000	117	+	+
CD2ΔC98	trans ⁺	10,000	98	+	+
CD2ΔC43	trans [−]	5,000	43	—	—

Production and characterization of 3DO54.8 transfectants expressing either the wild-type human CD2 (CD2FL) or partially deleted CD2 molecules containing the entire extracellular and transmembrane segments of CD2 but only 98 (CD2ΔC98) or 43 (CD2ΔC43) residues of the 117-amino acid long cytoplasmic domain, respectively, is described in detail elsewhere¹³. In brief, to construct the truncated CD2 molecules, the human CD2 cDNA, PB2 (ref. 20), was digested with restriction enzymes *FokI* and *AvaI* and blunted with T4 DNA polymerase. The *FokI* and *AvaI* digests were ligated to the linker 5'-TAAGGATCCTTA-3' to introduce a termination codon and provide a *BamHI* restriction recognition site. Subsequently, the DNAs were inserted into the *BamHI* site of the DOL vector (provided by Dr Thomas Roberts). Plasmids containing CD2 cDNAs were isolated and sequenced around the modified region by double-stranded sequencing before Ca²⁺ precipitation and transfection into ψ-2, a helper-free retrovirus packaging cell line. Both transiently expressed and permanent viral stocks were used to infect the murine T-cell hybridoma, 3DO54.8 (ref. 21) (provided by P. Marrack, National Jewish Hospital) in the presence of 8 μg ml^{−1} polybrene (Aldrich Chemicals). Selection was initiated 48 h after infection using 0.4 mg ml^{−1} G418 (Geneticin, Gibco) and wells containing single colonies expanded. G418 resistant clones reactive with murine anti-human CD2 antibody were sorted on the Epics V cell sorter. Cells were maintained in RPMI 1640 (Gibco) medium supplemented with 10% heat-inactivated fetal calf serum (Flow Labs), 50 μM 2-mercaptoethanol (2-ME), 1 mM sodium pyruvate, 2 mM L-glutamic acid, 1% penicillin-streptomycin (Whittaker) and 0.4 mg ml^{−1} G418. Stimulation of CD2FL or CD2ΔC98 cells with anti-T11₂ + anti-T11₃ mAbs results both in cytosolic calcium rise and IL-2 production whereas these antibodies fail to activate CD2ΔC43 cells¹³. Therefore, the designation trans⁺ or trans[−] refers to the capacity of CD2 molecules to transduce or fail to transduce activation signals, respectively. Approximate number of copies per cell was determined by quantitative immunofluorescence relating fluorescence channels to copy numbers of cells whose quantitation was determined by radiolabelled 3T4-8B5 anti-T11₁ antibody binding studies. NA, not applicable.

in antigen processing by either B-cell line. Individual 3DO54.8 transfectants produce differing but reproducible amounts of IL-2 after ova + A20 antigen-presenting cell stimulation, reflecting interclonal variability. More importantly, all transfectant cell lines expressing the CD2 extracellular segment, including CD2 Δ C43, produce substantially more IL-2 when stimulated by ova and the P24A20 antigen-presenting cells, as opposed to the A20 antigen-presenting cells: 350%, 800% and 400% for CD2FL, CD2 Δ C98 and CD2 Δ C43, respectively. The relatively greater increase shown by CD2 Δ C98 apparently correlates with the higher level of human CD2 expression on this cell line. LFA-3 expression on the antigen-presenting cell is by itself, not sufficient to augment T-cell responses, as P24A20 has no augmenting effect on IL-2 production by 3DO54.8 cells that do not express human CD2. Furthermore, the observed augmentation of IL-2 production is linked to the transfected human CD2 and LFA-3 cDNAs, as reduction of IL-2 secretion to levels stimulated by ova + A20 antigen-presenting cells (Fig. 2c, right panel) is obtained when CD2FL, CD2 Δ C98 or CD2 Δ C43 (not shown) are stimulated by ova + P24A20 in the presence of anti-T11₁ or LFA-3 monoclonal antibodies. These antibodies, however, are without effect on the stimulation of 3DO54.8 cells with ova + P24A20 (data not shown). The results presented in Fig. 2c (left panel) were obtained using a suboptimal concentration of ovalbumin (0.5 mg ml⁻¹). Increases in IL-2 production (150–300% of control) were also obtained using high (2 mg ml⁻¹) or low (0.06 mg ml⁻¹) concentrations of antigen.

We suspect that our results underestimate the enhancement of antigen response affected by the human CD2-LFA-3 adhesion pair as it is likely that murine CD2 and LFA-3 are present on 3DO54.8 and A20, respectively. Direct analysis using a rat

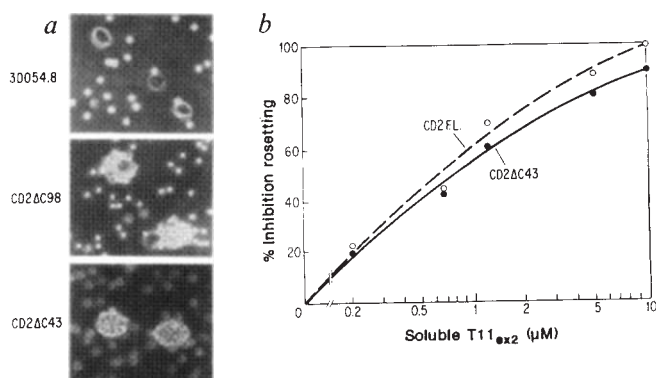


FIG. 1 Adhesion function of CD2 and CD2 cytoplasmic truncation variants is unchanged. **a**, Rosetting between human CD2-transfected murine T cells and sheep red blood cells (SRBC). Percentage of cells forming rosettes are 0%, 67%, 97%, 70% for 3DO54.8, CD2FL, CD2 Δ C98, CD2 Δ C43, respectively, and correlate with the level of staining with the anti-T11₁ monoclonal antibody (mAb) 3T4-8b5 (not shown). **b**, CD2 affinity measurement using a rosette inhibition assay with increasing concentration of soluble T11_{ex2} protein. T11_{ex2} is a recombinant monomeric soluble CD2 molecule of relative molecular mass 30,000 that corresponds to protein encoded by the two extracellular segment exons and contains the LFA-3 binding site, as well as the T11₁, T11₂ and T11₃ epitope¹⁴. A concentration of 10 μ M corresponds to 300 μ g T11_{ex2} per ml.

METHODS. **a**, SRBC were preincubated for 15 min at 37 °C with aminoethyl isothiuronium bromide (AET) (Sigma), washed three times and resuspended (5% v/v) in Hank's buffered salt solution (HBSS) + 2% FCS. Ten μ l of this SRBC suspension were added to 10⁵ 3DO54.8 transfectants in a final volume of 80 μ l. Tubes were incubated for 5 min at 37 °C, spun down for 5 min at 800 r.p.m. and incubated for 1 h at 4 °C. After gentle resuspension, 200 cells were counted under a Zeiss light microscope and T cells bound to at least four SRBC were counted as rosette-positive. **b**, Ten μ l of a 5% v/v suspension of AET-treated SRBC were preincubated for 1 h at 4 °C in HBSS + 2% FCS containing various concentrations of T11_{ex2} (ranging from 0 to 10 μ M) before addition of CD2FL or CD2 Δ C43 cells (10⁵ cells per tube). Rosetting experiments were performed as above. Results are representative of three separate experiments.

anti-murine CD2 antibody¹⁶ (provided by K. Okamura) detected a low level of murine CD2 present on 3DO54.8. Because this antibody fails to block murine T-cell function, it could not abrogate any putative murine T-B cell interaction. Likewise, that there is not a greater enhancement of IL-2 production when the CD2_{trans}⁺ clone CD2FL is stimulated by ova + P24A20 versus ova + A20 (350%) relative to similar triggering (400%) of the CD2_{trans}⁻ CD2 Δ C43 clone, might be a consequence of endogenous murine CD2-murine LFA-3 interactions having already provided signals which synergize with those resulting from antigen-triggered activation. This would not be the case

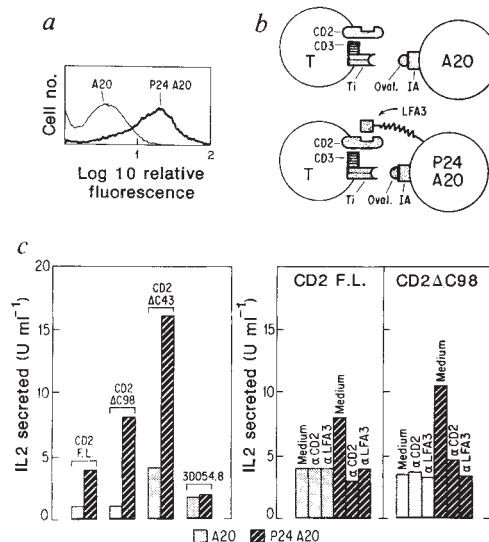


FIG. 2 Functional consequence of the CD2-LFA-3 interaction during antigenic stimulation. **a**, Flow cytometric analysis of human LFA-3 expression on A20 and the transfected P24A20 murine B cells. Immunofluorescence assays were carried out with the Fab fragment of the anti-LFA-3 TS2/9 mAb (provided by Dr T. Springer, Harvard Medical School) directly conjugated with fluorescein isothiocyanate (FITC). **b**, Schematic representation of cell-cell interactions. **c**, Quantitation of IL-2 production by CD2-transfected murine T cells stimulated with antigen-pulsed A20 or P24A20 B cells.

METHODS. **a**, P24A20 cells were derived from the I-A^d A20 B lymphoma by transfection with 100 μ g *Pvu*I-linearized expression plasmid pJOD-S-P24 LFA-3-neo, which contains the full-length cDNA for the phosphatidylinositol-linked form of human LFA-3, as well as the neomycin-resistance gene (ref. 17 and B.W., manuscript in preparation). Transfectants were obtained by electroporation with Biorad gene pulse (Richmond at 0.20 μ V with capacitance set at 960 μ F, and selected in culture medium (RPMI 1640 + 10% FCS, 1 mM sodium pyruvate, 2 mM L-glutamine, 1% penicillin-streptomycin and 50 μ M 2-mercaptoethanol) supplemented with 1 mg ml⁻¹ G418. Neomycin-resistant clones were further selected for LFA-3 surface expression by direct immunofluorescence analysis (10,000 cells per sample) using Fab fragments generated by papain digestions according to manufacturer recommendations (Pierce) and directly conjugated²² with FITC. LFA-3 surface expression on P24A20 cells is estimated to be 45% of that of the human B cell line JY surface LFA-3 expression. Although not shown, the A20 cell line and P24A20 express identical levels of the I-A^d gene product as judged by indirect immunofluorescence with the anti-I-A^d monoclonal antibody MKD6 (provided by L. Glimscher, Harvard School of Public Health). **c**, CD2-expressing cells (10⁵ per well) were incubated in 96-well round bottom plates for 24 h in the presence of either A20 or P24A20 cells (50,000 cells per well) pulsed with ovalbumin (0.5 mg ml⁻¹ final concentration). Supernatants were harvested and serial twofold dilutions titrated in triplicate for their ability to support the growth of 10,000 cells of the IL-2-dependent CTLL2 line. Cultures were pulsed after 24 h incubation with 1 μ Ci ³H-thymidine per well and harvested after overnight incubation on a Mash apparatus. Filters were dried and after addition of scintillation fluid counted on a beta counter. Results represent the mean of triplicate determinations (with standard deviations <10%) of IL-2 production, quantitated by running culture supernatants in parallel to a titration curve of recombinant IL-2 of known specific activity (Biogen) and are representative of three independent experiments. In blocking experiments, anti-T11 (3T48B5 ascites at a 1:400 dilution) or anti-LFA-3 (supernatant at a 1:6 final concentration) mAbs were added at the initiation of the assays.

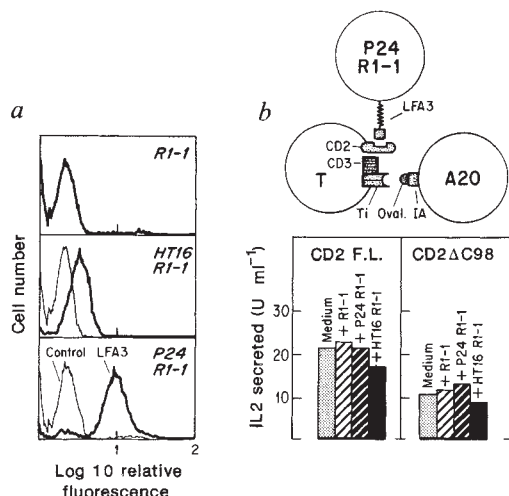


FIG. 3 Co-stimulation of CD2 transfected murine T cells with antigen-pulsed A20 cells plus LFA-3 expressing third party cells: requirement for LFA-3 expression on the cognate partner. *a*, Immunofluorescence analysis of human LFA-3 expression on transfected thymoma. P24R1-1 and HT16R1-1 were derived from the murine thymoma R1-1 by transfection with the full-length human LFA-3 cDNA encoding the phosphatidyl inositol-linked and transmembrane forms of the molecule, respectively, as described in Fig. 2. *b*, IL-2 production by CD2⁺ transfectants upon antigenic stimulation in the presence or absence of third party cells.

METHODS. *a*, Cells were stained for 30 min at 4 °C with the anti-LFA-3 TS2/9 mAb (supernatant at a 1:5 final dilution), washed twice, and incubated with goat anti-mouse IgG second antibody (1:40 dilution, Meloy). Cells were analysed (10,000 per sample) on an Epics V cell sorter. Histograms represent reactivity with anti-LFA-3 MAb (thick line) compared with an equivalent amount of an irrelevant antibody (1HT4 4E5 ascites, 1:200 final dilution) (thin line). *b*, IL-2 assays were performed as in Fig. 2c but included, in addition, 50,000 R1-1 or HT16R1-1 or P24R1-1 cells per well. IL-2 production resulting from stimulation of 10⁵ CD2 transfectants with 50,000 A20 cells plus ovalbumin alone are represented in the bar labelled 'medium'.

for CD2-LFA-3 interactions in cellular systems where the stimulus for the T cell is presented on a xenogeneic cell population (which fails to bind the endogenously expressed CD2 of the other species), or liposomes bearing purified alloantigen and LFA-3 molecules⁸. These latter systems have readily identified the synergistic effect of CD2-LFA-3 signal transduction on T-cell receptor triggered activation. In contrast to the CD2 adhesion function defined here, CD2-mediated signal transduction requires the CD2 cytoplasmic tail^{8,12,13}.

The augmented IL-2 production observed after stimulation of human CD2⁺ murine T-cell clones by antigen and human LFA-3-expressing antigen-presenting cells probably results from the ability of the CD2-LFA-3 adhesion pair to facilitate more efficient formation of the ternary complex between the T-cell receptor and antigen plus the MHC gene product. Although the affinity of monomeric CD2-LFA-3 interactions is low (micromolar) the increased avidity resulting from multimeric interactions of multiple transmembrane CD2 copies on a given T cell with multiple LFA-3 molecules on the antigen-presenting cell is likely to be great and should serve to oppose, more optimally, surface membranes of the two cell types. Alternatively, if the interaction between LFA-3 and CD2 provides an as yet unrecognized signal that enhances IL-2 responsiveness independently of the known CD2 signalling mechanisms, then an LFA-3-CD2 interaction resulting from an unrelated third-party cell might enhance the functional consequences of T-cell receptor triggering, thereby resulting in enhanced IL-2 production. To test this possibility, cDNAs encoding the transmembrane form as well as the phosphatidyl inositol-linked form of LFA-3 were transfected into a thymoma cell line (R1-1), which does not express I-A^d, to yield cell lines termed HT16R1-1 and P24R1-1 respectively (ref. 17 and B.P.W., manuscript in preparation). As shown by immunofluorescence analysis (Fig. 3*a*), the phosphatidyl inositol-linked

LFA-3 form is expressed at levels about fivefold higher than the transmembrane form. Each of these cell lines was tested for their co-stimulatory effect on human CD2 expressing murine T cells in conjunction with ova pulsed A20 (schematically depicted in Fig. 3*b*). A representative experiment is shown in Fig. 3*b* (bottom). Identical results are found at higher and lower third-party cell numbers (not shown). No significant differences were observed in IL-2 production following stimulation by ova + A20 cells, regardless of which third-party cell was added into the assay. Similar results were obtained using the CD2ΔC43 and 3D054.8 IL-2 producing cells lines (data not shown). Thus, the adhesion mediated between CD2 and LFA-3 optimizes the physical interaction between the antigen-responsive T cell and its cognate partner.

Earlier studies using monoclonal antibodies directed against either the CD2 adhesion domain (anti-T11₁-like) or LFA-3 delineated a profound inhibition of helper and cytotoxic T-cell responses and thymocyte proliferative responses in *in vitro* assays^{9,10,18}. The present results unambiguously define the importance of CD2-LFA-3 adhesion in such interactions even in the absence of CD2 signal transduction. Thus, the CD2-LFA-3 adhesion pair, like the CD4-MHC class II adhesion pair¹⁹, augments T-cell response even in the absence of their respective CD2 or CD4 cytoplasmic segments. We presume that CD2 and LFA-3 function similarly to increase the interactions between a cytolytic T lymphocyte and its target cell. One can imagine that this adhesion is also important for thymocyte-thymic epithelial interactions involved in thymic education and differentiation. □

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Rapid induction of neutrophil-endothelial adhesion by endothelial complement fixation

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THE adhesion of neutrophils to vascular endothelium is an early event in their recruitment into acute inflammatory lesions¹. In evaluating potential neutrophil-endothelial adhesive mechanisms in acute inflammation, important considerations are that adhesion

in vivo may occur very rapidly following injury²⁻⁴ and that the specificity of the reaction resides in altered endothelium⁵. That is, neutrophils adhere only to altered endothelium adjacent to an inflammatory focus, rather than at random as would be expected if activation of neutrophils were the initiator of adhesion. We have explored a possible bridging role for complement in causing early neutrophil-endothelial cell adhesion. The complement system is involved in inflammatory processes, is capable of rapid amplification, and endothelial complement fixation at sites of inflammation could generate an endothelium-restricted signal for neutrophil adhesion. We have now developed a model in which this can be investigated without complicating factors such as immunoglobulin deposition, by constructing a novel molecule, a hybrid of the endothelial binding lectin *Ulex europaeus* I (ref. 6) and of the complement activator cobra venom factor^{7,8}. This molecule has the capacity to cause fixation of complement on human umbilical vein endothelial cells. We show that complement fixation is a potent and rapid stimulus for neutrophil adhesion. Neutrophil adhesion requires only endothelial deposition of C3, and is mediated through the type 3 complement receptor.

A hybrid of *Ulex europaeus* I (Ulex) and cobra venom factor (CVF) was constructed using the heterobifunctional cross-linking compound *N*-succinimidyl 3-(2-pyridyldithio)propionate. The hybrid molecule, referred to as Ulex-CVF was radioiodinated for tracing purposes and shown to retain the Ulex lectin property of time and dose saturable binding to human umbilical vein endothelial cells, as well as retaining one third of the native CVF complement-activating activity, as assessed by a haemolytic assay (not shown). Pretreating endothelial cells with Ulex-CVF followed by serum as a complement source induced surface deposition of complement. Endothelial-bound iC3b, C5, and the membrane attack complex (MAC) were detected by radioimmunoassay (Fig. 1).

Treating endothelial cells with Ulex-CVF followed by serum resulted in a high degree of neutrophil adhesion. In Fig. 2 the

time and dose relationships for the induction of adhesion are demonstrated. The effect was detected within 1 min of adding the complement source and a high proportion of the cells added could be induced to adhere (approaching 100%). The adhesion reached a peak within 20 min and was maintained for up to 8 hours. Removing the source of complement led to the rapid decay of adherence (not shown).

The time course of adhesion contrasts strongly with that caused by treating endothelial cells with the cytokine tumor necrosis factor α (TNF), a previously defined mechanism for inducing neutrophil-endothelial adhesion⁹. Endothelial cells stimulated with TNF at a concentration of 200 U ml⁻¹, (human recombinant, 1 ng = 10 U, Cetus) had only basal levels of adhesion after 30 minutes stimulation (77% of control versus 1,869% for Ulex-CVF and serum treated cells), a minimal increase after 1 h of stimulation (143% of control adhesion) and only developed a marked increase at 2 h after stimulation (408% of control adhesion). There is also an interesting contrast between the strength of the adhesion induced by complement and other adhesion mechanisms. Using the same washing conditions to remove unbound neutrophils we could barely detect the adhesion commonly studied by activating neutrophils with

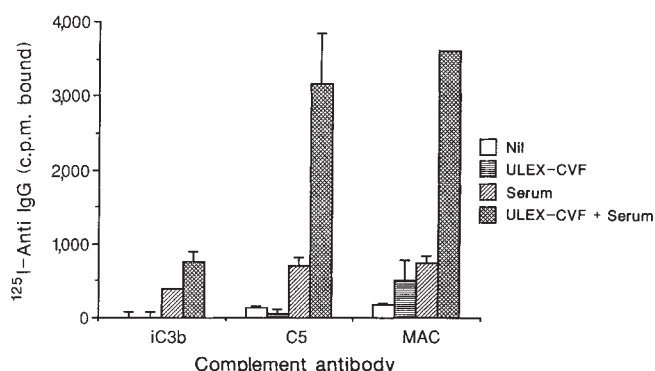


FIG. 1 Induction of endothelial complement deposition by Ulex-CVF. The use of the surface bound complement activator Ulex-CVF followed by human serum as a complement source was associated with the formation of iC3b in the endothelial membrane and binding of C5 and the membrane attack complex. The use of serum alone was associated with some complement fixation but always less than that associated with use of the activator.

METHODS. Endothelial cells were extracted and cultured from human umbilical veins according to the method of Jaffe²⁹. Cells were cultured in 20% fetal bovine serum (Hyclone) in medium 199 (Gibco) supplemented with 100 μ g ml⁻¹ endothelial cell growth supplement (Collaborative Research) and 100 μ g ml⁻¹ bovine lung heparin (Sigma). Cells used in this and other experiments were derived from the second to fourth passages. Endothelial cells were seeded at 10,000 cells per 6.4 mm diameter microwell (Corning) previously coated with 10 μ g ml⁻¹ fibronectin (Calbiochem) for 10 min and allowed to grow to confluence for at least 2 days before being used. Confluent monolayers of cells in 96 well plates were washed and exposed to 50 μ l of 1.76 μ g ml⁻¹ Ulex-CVF or diluent alone for 2 h at 4 °C. The wells were washed 3 times with 100 μ l volumes and then 100 μ l of 80% fresh normal human serum or diluent alone was added for 30 min at 37 °C. The wells were washed 3 times and then either a mouse monoclonal antibody to a human iC3b neoantigen (Cytotech) or rabbit antibodies to human C5 (Calbiochem), or the membrane attack complex (MAC, Calbiochem) added for 1 h at 4 °C. The wells were washed 3 times and then either ¹²⁵I-labelled F_{ab2} fragments of sheep anti-mouse immunoglobulin antibody (622 ng ml⁻¹, 9 Ci g⁻¹ DuPont) or donkey anti-rabbit immunoglobulin antibody (144 ng ml⁻¹, 6 Ci g⁻¹, Amersham) were added for 1 h at 4 °C. The wells were washed 7 times and residual radioactivity released with 10% SDS and counted. The medium used for dilution and washing was phosphate buffered saline with calcium and magnesium (Gibco) and 0.5% w/v human serum albumin (Cutter) (PBS-HSA), supplemented with 0.02% sodium azide after the addition of antibodies. The results are presented as the mean c.p.m. associated with the use of each antibody minus the c.p.m. associated with use of the appropriate isotype or serum control. Error bars, standard deviation.

TABLE 1 Effect of antibodies to complement receptors and related molecules on neutrophil adhesion to complement-treated endothelium

Antibody	Reference	Specificity	Adhesion inhibition (%)
3D9	21	CR1	0
1B4	21	CR1	0
57F	22	CR1	0
17aba	23	CR3 α (CD11b)	71
44aac	23, 24	CR3 α	75
MN-41	25	CR3 α	65
KB90	26	CR4 α (CD11c)	5
L29	26	CR4 α	0
SPV-L7	27	LFA1 α (CD11a)	0
SPV-L11	27	LFA1 α	0
IB4	28	β -chain (CD18)	89
10F12		β -chain	78

Human umbilical vein endothelial cells were exposed to Ulex-CVF (1.76 μ g ml⁻¹) for 2 h at 4 °C followed, after washing, by 80% human serum for 30 min at 37 °C, as described in Fig. 1. Antibody was added to the endothelial cells in 50 μ l-phosphate-buffered saline (PBS) followed by another 50 μ l containing 2×10^5 ⁵¹Cr-labelled neutrophils. Neutrophil preparation and assessment of adhesion were as described in Fig. 2. Results are expressed as % inhibition of adhesion calculated as adhesion with antibody subtracted from adhesion without antibody and divided by adhesion without antibody. Isotype controls had no effect on adhesion and none of the antibodies had any effect on adhesion in the absence of endothelial complement deposition (not shown). The specificity of the antibodies is described in the references given in column two. 10F12 is a common β -chain (CD18) antibody (J. Ritz, personal communication). IB4 does not inhibit binding of iC3b coated erythrocytes to monocytes but it does block this interaction with neutrophils (S. D. Wright, personal communication). All antibodies were in the form of ascites and were used at 1/50 dilution.

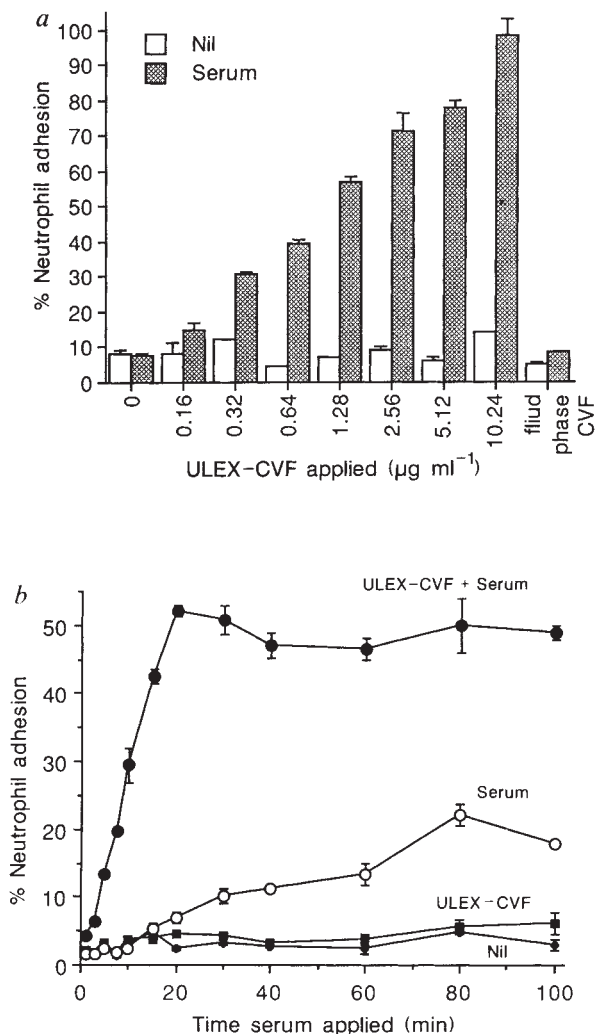


FIG. 2 Induction of neutrophil adhesion by endothelial complement deposition. *a*, Increasing neutrophil adhesion with increasing concentrations of Ulex-CVF followed by the addition of serum as a complement source. The ^{125}I -labelled Ulex-CVF was assessed over a 64-fold range of concentration. Fluid phase CVF refers to the addition of free CVF (Cytotech) at 10 U ml^{-1} with serum, as opposed to endothelial bound CVF, and indicates that fluid phase CVF is not able to substitute for surface bound CVF. *b*, Increasing neutrophil adhesion with increasing serum exposure time. There is some neutrophil adhesion with the use of serum alone, an effect variably present in other experiments, but much greater adhesion when the cells were pretreated with Ulex-CVF. In these and other experiments Ulex-CVF alone had no adhesive effect.

METHODS. Neutrophils were isolated by density gradient centrifugation (endotoxin free Lymphoprep, Nycomed) of citrated whole blood followed by two cycles of hypotonic lysis of the erythrocyte pellet. The final preparation was >95% polymorphonuclear and >95% viable. Neutrophils were labelled with $100 \mu\text{Ci}$ of ^{51}Cr sodium chromate (DuPont) in PBS without calcium or magnesium (Gibco) with gentle shaking for 1 h at 37°C , washed 3 times with PBS without calcium or magnesium and 0.1% HSA. Radioactivity remained >90% cell associated when assessed at the end of the adhesion assay. Endothelial cells in microwells (prepared as described in Fig. 1) were treated with PBS-HSA containing *a*, increasing amounts of Ulex-CVF or *b*, $1.76 \mu\text{g ml}^{-1}$ Ulex-CVF for 2 h at 4°C followed by 3 washes with PBS-HSA. Eighty per cent fresh human serum in PBS-HSA or PBS-HSA alone was then added, incubated with the cells at 37°C , and removed by washing 3 times with PBS-HSA after *a*, 30 min or *b*, at various intervals between 1 and 100 min. After removing the serum 2×10^5 ^{51}Cr -labelled neutrophils in $100 \mu\text{l}$ of PBS were added for 15 min at 37°C . Unattached cells were then removed by 3 washings of $150 \mu\text{l}$ of PBS, and the radioactivity associated with the bound neutrophils was released with 10% SDS and counted. Percentage adhesion was calculated by dividing the adherent counts by the total counts added per well. ^{125}I -labelled Ulex-CVF did not register any radioactivity when counting ^{51}Cr to assess adhesion. Error bars, standard deviation.

phorbol myristate acetate, formyl-Met-Leu-Phe or calcium ionophore A23187. In further studies, neutrophils maintained in continuous motion were arrested by and bound to complement treated endothelium but did not bind to contiguous control endothelium (not shown), suggesting that this adhesion may occur with sufficient avidity to be relevant to blood flow through the microvasculature.

The neutrophil receptor responsible for adherence to the complement treated endothelium was identified using a range of monoclonal antibodies specific for individual receptors and related molecules (Table 1). Unequivocal results identifying the type 3 complement receptor (CR3) as the responsible molecule were obtained. Antibodies to the α -chain of CR3 (CD11b) as well as antibodies to the β -chain (CD18) it shares with the other CD11 molecules¹⁰ strongly inhibited adhesion. Neither antibodies to the other CD11 molecules (CD11a, CD11c) nor antibodies to CR1 inhibited adhesion.

The finding that neutrophil CR3 was responsible for the binding suggested that iC3b, the only known complement factor which binds CR3 (ref. 11), was the endothelial-bound, ligand. This was investigated using purified complement components and reconstituting the alternate pathway to the extent necessary to induce neutrophil adhesion. It was found that factors B, D and C3 were all necessary and adequate for reconstitution. C3 was used at a concentration equivalent to 17% of that found in normal serum and was associated with a degree of adhesion similar to that caused by 25% serum (Fig. 3). Addition of later complement components as well as the regulatory components H and I led to no further increase in adhesion (not shown). Although recent work has suggested that neutrophil CR3 also binds fibrinogen¹² and coagulation factor X (ref. 13) in addition to iC3b, the reconstitution experiments demonstrated that the complement products were responsible for inducing adhesion. They further indicated that induction of adhesion was independent of the membrane attack complex and any associated injury. Complement factors B, D and C3 and magnesium in the presence

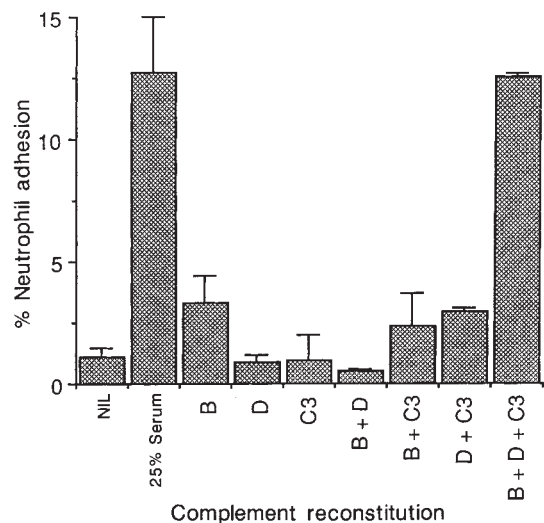


FIG. 3 Reconstitution of neutrophil adhesion with purified complement components B, D and C3 in comparison with the effect of serum. Cells in microwells were treated with PBS-HSA containing $1.76 \mu\text{g ml}^{-1}$ Ulex-CVF for 2 h at 4°C followed by 3 washes with PBS-HSA. Then either 25% fresh human serum or isolated complement components (Cytotech) were added in $40 \mu\text{l}$ of PBS-HSA for 30 min at 37°C . After 3 washes with PBS-HSA, neutrophils were added and adhesion assessed as in Fig. 2. All complement components were shown to migrate as a single species of the appropriate molecular weight on nonreducing SDS polyacrylamide gel electrophoresis. The concentrations used were B, $100 \mu\text{g ml}^{-1}$; D, $1 \mu\text{g ml}^{-1}$; C3, $200 \mu\text{g ml}^{-1}$. C3 was mixed with the other factors immediately before use. Neither serum nor the complement factors when used in the absence of Ulex-CVF had any effect on neutrophil adhesion (not shown). Error bars, standard deviation.

of an activator result in the formation of C3b (ref. 14). Further studies with ^{125}I -labelled C3, B and D, which assessed the formation of C3b and iC3b by following changes in molecular weight of the C3 α -chain with gel electrophoresis, demonstrated formation of iC3b from C3b (not shown). Although we cannot exclude possible factor I contamination of the isolated factors as a cause of iC3b formation, endothelial cells have detectable transcripts for factors H and I (ref. 15) and it seems likely that endothelium has the endogenous capacity to form iC3b from C3b.

Recent work has demonstrated that cytokine-stimulated endothelium becomes adhesive for leukocytes^{9,16}, a process requiring synthesis of membrane proteins such as ELAM-1 (ref. 17) and ICAM-1 (ref. 18). Adhesion is not detected until considerably after endothelial stimulation and peaks at ~4 h (ref. 9 and unpublished results). The rapidity with which adhesion may occur *in vivo* in response to various stimuli²⁻⁴ suggests that another mechanism preceding the more slowly developing cytokine-mediated induction of neutrophil-endothelial adhesion may play a role in acute inflammation. Our work demonstrates an additional and novel mechanism by which neutrophil adhesion to the vessel wall may be mediated. Vascular complement deposition in experimental and clinical inflammatory conditions is commonly observed¹⁹. We suggest that complement activation occurring in the context of an acute inflammatory stimulus adjacent to a vessel wall may cause endothelial complement deposition. It is also possible that an alteration in the susceptibility of the vessel wall to the fixation and subsequent degradation of complement may occur. The mechanism of how this could occur is unclear. It is of interest, however, that the molecule GMP-140, which is related to ELAM-1, is fused into the endothelial plasma membrane very rapidly following an activating stimulus. GMP-140 has sequence homology with proteins that have the common feature of being regulators of complement activation²⁰.

The important bound complement component is C3 which is converted to iC3b, the ligand of the neutrophil type 3 complement receptor. We noted somewhat less inhibition of adhesion using antibodies to the CR3 α -chain (CD11b) than with antibodies to the β -chain that CR3 has in common with CD11a and CD11c. This suggests at least the possibility of accessory interactions involving these molecules and other ligands. However this does not occur in the absence of prior complement fixation. The important aspects of this mechanism are that it develops rapidly, forms a strong neutrophil-endothelial bond, and retains the endothelial specificity that is such a marked feature of adhesion *in vivo*. □

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Pulsatile intracellular calcium release does not depend on fluctuations in inositol trisphosphate concentration

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MANY hormones, neurotransmitters and growth factors evoke in their target cells oscillations in the free internal Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) (ref. 1). In electrically non-excitable cells these fluctuations are due to periodic release of Ca^{2+} from intracellular reservoirs^{1,2}, stimulated by the internal messenger inositol trisphosphate (InsP_3) (refs 2-4). Most models at present invoke fluctuating levels of InsP_3 as a key component in generating the oscillations in $[\text{Ca}^{2+}]_i$ (refs 5 and 6). InsP_3 injected into intact cells evokes irregular and transient oscillatory Ca^{2+} -dependent current responses⁷⁻⁹, but the intracellular InsP_3 concentration is not constant in such experiments. Here we monitor changes in $[\text{Ca}^{2+}]_i$ by measuring Ca^{2+} -activated Cl^- current in single internally perfused mouse pancreatic acinar cells^{4,10-12} and show that acetylcholine (ACh), acting through muscarinic receptors, evokes regular and repetitive current pulses which are mimicked by InsP_3 applied through a patch pipette¹³. To exclude the possibility that InsP_3 is periodically phosphorylated¹⁴ or degraded, we replaced it by the non-metabolizable InsP_3 analogue inositol trisphosphorothioate (InsP_3S)^{9,15,16}, which also evokes regular pulses of Ca^{2+} -activated Cl^- current. These effects are independent of external Ca^{2+} , but abolished by high intracellular concentrations of a Ca^{2+} -chelator. We conclude that repetitive pulses of intracellular Ca^{2+} release occur even when the concentration of InsP_3 is constant.

Figure 1a shows that 0.1 μM ACh evokes regular pulses of inward current. In this experiment there were approximately 3 pulses per min, although there was variation from cell to cell (2-6 per min) and the duration of the pulses ranged from ~3-18 s. This pattern was obtained in nine experiments. In one experiment there was no ACh effect whereas in two other experiments a sustained non-oscillating response was observed. The frequency range of the stimulant-evoked current pulses at room temperature is similar to that of the $[\text{Ca}^{2+}]_i$ fluctuations observed in intact mouse pancreatic acinar cells¹⁷ (3-4 per min, at 30 °C), parotid acinar cells¹⁸ (6-16 per min, at room temperature), liver cells¹⁹ (1 per 2 min-2 per min, at 37 °C) and neutrophils²⁰ (1-2 per min, at 37 °C). At 1 μM , ACh normally evoked an oscillating response, as seen in Fig. 1b, but the current did not return to the baseline between the peaks. At 10 μM , ACh evoked a smooth sustained increase in the inward current (Fig. 1c). All effects of ACh were abolished by atropine (Fig. 1a, c) ($n = 13$) as well as by including a high concentration of the Ca^{2+} -chelator EGTA in the pipette filling solution (Fig. 1d) ($n = 5$). The effect of ACh was independent of the presence or absence of external

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Ca^{2+} (Fig. 1e) ($n=7$) during the first 7–8 minutes after start of stimulation. In later periods extracellular Ca^{2+} dependence became apparent in some experiments, but the responses were mostly lost before that happened. The presence of GTP- γ -S (50–100 μM) greatly enhanced the effect of low ACh doses²¹ or, by itself, induced current responses similar to those evoked by ACh. In the concentration range 0.1–10 μM , the ACh effect was graded not so much by changing the maximal amplitude of the inward current, but mostly by increasing the time during which current flowed (Fig. 1f). This result is consistent with previous data which suggests that the Ca^{2+} -signalling system is frequency-encoded rather than being amplitude dependent^{1,2,19}.

That the ACh-evoked current was carried by Cl^- is indicated by the fact that the null (or equilibrium) potential during ACh application varied linearly with the Nernst potential when this was changed from approximately 0 to -45 mV (Fig. 1g). It is generally agreed that ACh can open both Ca^{2+} -activated Cl^- and Ca^{2+} -activated non-selective cation channels in the mouse and rat pancreatic acinar cells^{10–12}. However, unlike acinar cells from most other exocrine glands in various species, there is no activation of Ca^{2+} -dependent K^+ -selective channels²². The Cl^-

channels are opened at $[\text{Ca}^{2+}]_i$ of $\sim 1 \mu\text{M}$, whereas the non-selective channels require 10 μM (ref. 12). Furthermore, the Ca^{2+} -activated non-selective cation channels in acinar cells are closed by intracellular ATP (ref. 23): thus, as high concentrations of ATP were present in our pipette solutions, ACh increased only the Cl^- conductance.

Intracellular application of 10 μM InsP_3 (inositol 1,4,5-trisphosphate) evoked regular pulses of Cl^- current, in 13 out of 16 experiments, similar to those evoked by 0.1 μM ACh (Fig. 2c, d). At the lower InsP_3 concentration of 1 μM , smaller oscillations were occasionally seen (Fig. 2b) (2 out of 5 experiments), whereas 100 μM InsP_3 evoked smooth and sustained increases in the Cl^- current (Fig. 2e) ($n=8$). The effects of InsP_3 , like those of ACh, were abolished by a high intracellular EGTA concentration (Fig. 2f) ($n=4$). Inositol 1,3,4-trisphosphate ($\text{Ins}(1,3,4)\text{P}_3$) (10 μM) evoked no increase in Cl^- current in 8 out of 11 experiments (Fig. 2a), whereas transient stimulations were seen in the three remaining cases. The effects of InsP_3 were independent of the presence or absence of external Ca^{2+} (Fig. 2g) ($n=6$) during the first 7–8 min after establishment of the whole-cell current recording configuration.

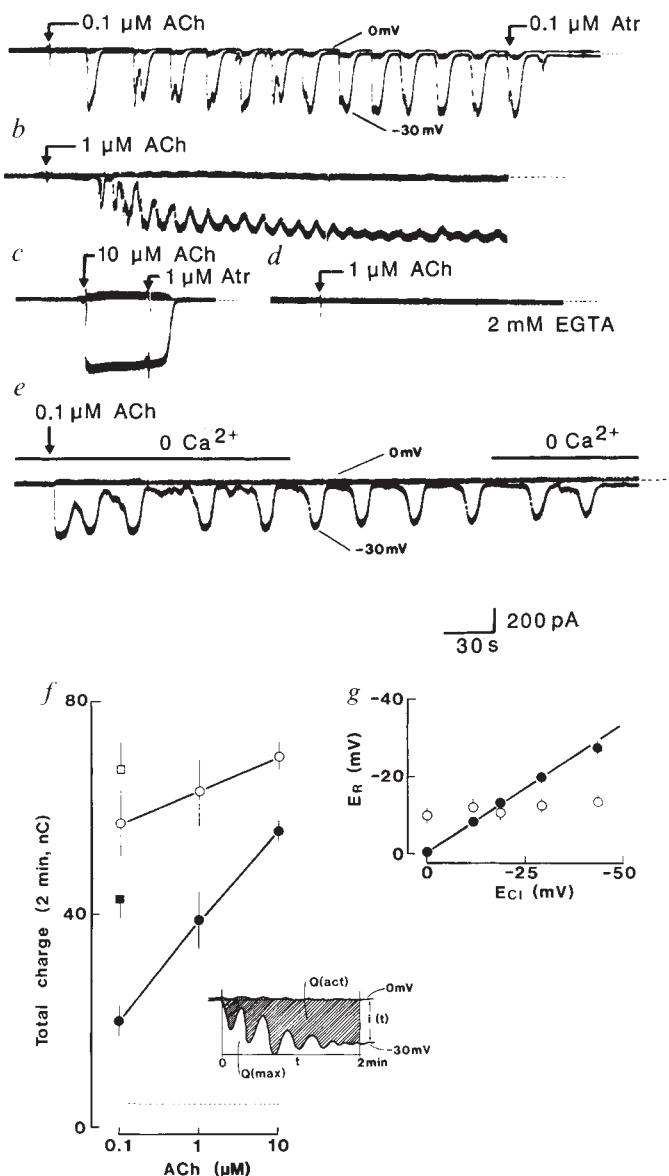


FIG. 1 Effects of acetylcholine (ACh). Traces of whole-cell current from single mouse pancreatic acinar cells. Acinar cells were voltage-clamped at a holding potential of -30 mV and depolarizing voltage jumps of 100-ms duration to a membrane potential of 0 mV were repetitively applied throughout all experiments. For these experiments Cl^- equilibrium potential (E_{Cl}) is ~ 0 . Because of compression of pen-recording traces, all records seem to show currents at -30 and 0 mV simultaneously. Dotted lines indicate zero current level. Effects of ACh (0.1 to 10 μM) are shown in a, b and c. In a and c, ACh response is abolished by atropine (Atr). In d a high intracellular EGTA concentration (2 mM) prevents ACh response. e shows that removal and readmission of external Ca^{2+} has no effect. ACh was applied ~ 2 min after establishment of whole-cell configuration. Latency between ACh application and initiation of response was generally shorter at 10 μM than at 0.1 μM . No clear differences between latencies with 0.1 and 1 μM ACh were noted. f shows dose-response curve for ACh-evoked Cl^- current expressed as charge movement during first 2 min of response. Boxes represent values obtained in presence of 50 μM GTP- γ -S. Filled symbols represent actual charge movement whereas open symbols represent theoretical maximal charge movement that would have occurred if maximal current amplitude had been maintained throughout the 2 min period (see inset) (mean values $\pm$ s.e., $n=6-11$). Horizontal dotted line shows basal total charge movement during 2 min, obtained from 8 experiments without ACh stimulation. g shows relationship between Nernst potential for Cl^- (E_{Cl}) (varying intracellular Cl^- concentration) and zero-current membrane potential (E_R) in absence (open circles) and presence (closed circles, line) of ACh (1 μM) ($n=7$ at each point).

METHODS. Fragments of mouse pancreas were digested by pure collagenase (Worthington, 200 units ml^{-1} , 20 min, 37°C) in the presence of 1 mM Ca^{2+} . After wash with physiological saline, cell suspension was gently pipetted to obtain further separation and then again washed with control solution. Only single cells were used for experiments. The tight-seal, whole-cell current configuration of the patch-clamp technique was used for the measurement of the transmembrane current from single cells¹³. The standard extracellular solution contained (mM): NaCl 140, KCl 4.7, CaCl_2 1.0, MgCl_2 1.13, HEPES 10 and glucose 10. In Ca^{2+} -free solutions, CaCl_2 was omitted and 0.2–0.5 mM EGTA added (pH 7.2). In the pipette solutions K^+ concentration was fixed at 140 mM, whereas Cl^- concentration varied from 30 to 142 mM (replacement with sulphate, constant osmolality, sucrose balanced). EGTA concentration was normally 0.25 mM, but increased up to 10 mM as indicated when needed. No Ca^{2+} was added to the pipette solution, but 1.13 mM MgCl_2 and 5 mM Na_2ATP were present (pH 7.2). For intracellular application of inositol polyphosphates, the pipette was filled with normal pipette solution containing D-inositol 1,4,5-trisphosphate (InsP_3), D-inositol 1,3,4 trisphosphate ($\text{Ins}(1,3,4)\text{P}_3$) (both donated by Robin Irvine, AFRC Babraham, Cambridge) or D,L-inositol 1,4,5-trisphosphorothioate (InsPS_3) synthesized as previously described¹⁵. The concentrations of D,L- InsPS_3 are expressed as total concentrations, but the L-isomer is inactive^{9,16,24}. The active InsPS_3 concentration is therefore only half that indicated. All experiments were carried out at room temperature ($22-25^\circ\text{C}$).

The InsP_3 analogue inositol 1,4,5-trisphosphorothioate (InsPS_3) is only about threefold less potent than InsP_3 at releasing intracellular Ca^{2+} (refs. 9, 16, 24). It is, however, resistant to metabolism through the 5-phosphatase pathway^{16,25} as well as the 3-kinase pathway¹⁶ and is a potent inhibitor of 5-phosphatase²⁶. Microinjections of InsPS_3 into *Xenopus* oocytes evoked effects similar to those of InsP_3 ; these transient responses (lasting about 2 min) consisted of irregular oscillatory depolarizations⁹. As the InsP_3 or InsPS_3 concentrations in the cytosol in these experiments could not be constant, it was impossible to distinguish between transient ringing and a true autonomous oscillation. Figure 3 shows that a constant level of InsPS_3 can evoke repetitive pulses of Ca^{2+} -dependent Cl^- current. At $1 \mu\text{M}$, InsPS_3 did not evoke any response (Fig. 3a) ($n=4$). At $10 \mu\text{M}$, InsPS_3 evoked infrequent pulses of Cl^- current (Fig. 3b) ($n=6$), where at $30 \mu\text{M}$, regular current pulses of an amplitude and frequency similar to those evoked by $0.1 \mu\text{M}$ ACh or $10 \mu\text{M}$ InsP_3 were seen (Fig. 3c) ($n=6$). At $100 \mu\text{M}$, InsPS_3 evoked sustained and smooth responses (Fig. 3d) ($n=5$). The effect of InsPS_3 was, like that of ACh and InsP_3 , independent of the presence of external Ca^{2+} (Fig. 3e) ($n=6$).

It has been suggested that InsP_3 evokes oscillations in $[\text{Ca}^{2+}]_i$ (measured as Ca^{2+} -dependent Cl^- current) of a much higher frequency than those elicited by muscarinic receptor activation in rat lacrimal acinar cells²¹. However, current records in lacrimal acinar cells²¹, as well as liver cells²⁷, unlike the ones from the mouse pancreatic acinar cells shown here, never displayed continuous and regular InsP_3 evoked oscillations, making such a comparison difficult. In our experiments the ranges of oscillation frequencies obtained with ACh and InsP_3 as well as with InsPS_3 overlapped: ACh, 2–6 pulses per min; InsP_3 and InsPS_3 : 2–9 pulses per min).

Ever since Cobbold and his colleagues¹⁹ first showed that a constant agonist concentration could evoke regular repetitive spikes of $[\text{Ca}^{2+}]_i$, the mechanism has been sought. The receptor-controlled oscillator model proposes that the Ca^{2+} spikes are generated by spiking levels of InsP_3 (refs. 1, 2, 5, 6). Our data on the mouse pancreatic acinar cells do not support this hypothesis as constant levels of even the non-metabolizable inositol trisphosphate analogue InsPS_3 can readily evoke regular repetitive pulses of Ca^{2+} -activated Cl^- current with a pattern similar to that evoked by muscarinic receptor activation or by direct GTP- γ -S interaction with the transducing G-protein. Although evidence now suggests roles for InsP_4 and InsP_3 in agonist-evoked sustained elevations of $[\text{Ca}^{2+}]_i$ that are depen-

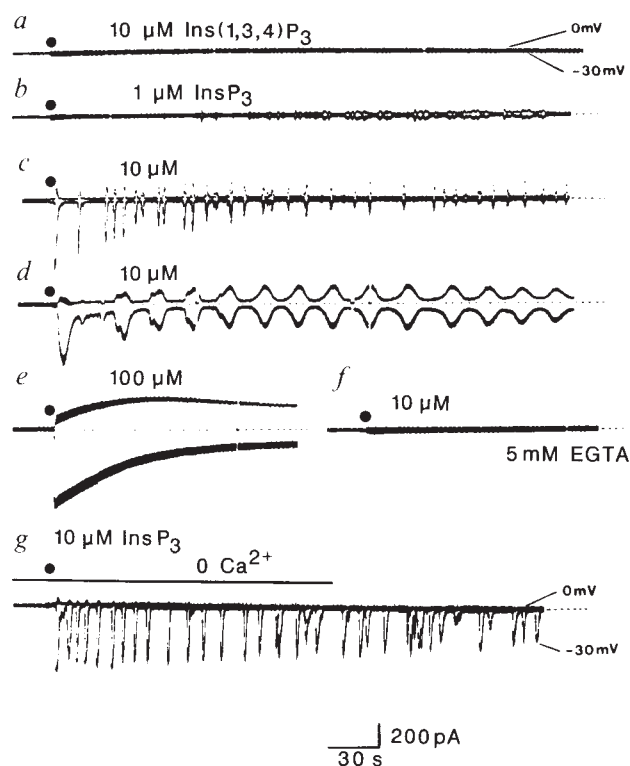
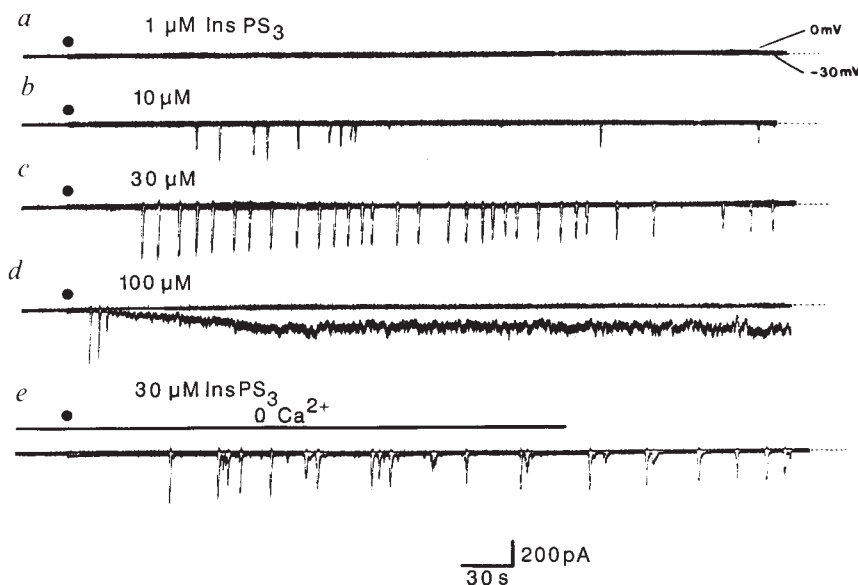


FIG. 2. Effects of InsP_3 (b–e) and lack of effect of $\text{Ins}(1,3,4)\text{P}_3$ (a). In a–f $[\text{Cl}^-]$ (in pipette solution) was $\sim 50 \text{ mM}$, whereas $[\text{Cl}^-]_o$ was $\sim 150 \text{ mM}$. Reversal potential during initial period after establishment of whole-cell configuration gradually changed to more negative values most likely due to change in E_{Cl^-} . Black dots close to beginning of traces indicate time points at which whole-cell recording configuration was established and therefore stimulation with inositol polyphosphate began. c and d, two types of responses to $10 \mu\text{M}$ InsP_3 . 7 out of 16 cases were of the type shown in c, whereas 6 out of 16 cases were of the type shown in d. f, the effect of InsPS_3 is blocked by high intracellular EGTA concentration. g, experiment in which E_{Cl^-} was close to 0 and in which removal and readmission of external Ca^{2+} had no effect on InsP_3 response.

FIG. 3. Effects of InsPS_3 . E_{Cl^-} was ~ 0 . InsPS_3 $1 \mu\text{M}$ had no effect a. At higher concentrations (10 – $100 \mu\text{M}$) InsPS_3 was effective in all cases examined (b–d). Response to $100 \mu\text{M}$ InsPS_3 consisted of a few initial spikes followed by a sustained increase (d). e, InsPS_3 effect in absence of Ca^{2+} (0.5 mM EGTA) outside cell showing lack of effect of readmitting external Ca^{2+} .



dent on extracellular Ca^{2+} (refs. 28, 29), our results argue against an important role for InsP_4 in the generation of $[\text{Ca}^{2+}]_i$ oscillations, because InsP_3 is apparently not metabolized by the 3-kinase pathway that leads to InsP_4 (ref. 16). The pulses of Ca^{2+} -activated Cl^- current are probably due to intermittent opening of Ca^{2+} channels in the intracellular reservoirs at a constant InsP_3 level or alternatively to periodic activation of the active process of Ca^{2+} accumulation. There may of course be more than one important mechanism of $[\text{Ca}^{2+}]_i$ oscillation. Further progress is likely to depend on more precise knowledge of the different types of intracellular Ca^{2+} pools, in which there seems to be at least two very different Ca^{2+} uptake mechanisms³⁰.

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Independent transfer of mitochondrial plasmids in *Neurospora crassa*

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IN the ascomycete fungus *Neurospora*, the distribution of homologous mitochondrial plasmid DNAs in different species¹ and among mitochondrial types² of *N. crassa* suggests that these molecules have moved between lineages of clonally propagated mtDNA. Here we report direct evidence for independent inheritance of mitochondrial plasmids by sexual reproduction which may help explain the distribution of these molecules among mitochondrial lineages.

To study the transmission of mitochondrial plasmid DNAs in *N. crassa* we took advantage of the prolific and rapid sexual reproduction of this species, maternal transmission of mtDNA^{3,4}

and the availability of maternally expressed ascocarp colour mutants that indicate parentage unambiguously.

We first investigated the distribution of mitochondrial plasmid DNAs in fifteen Louisiana *N. crassa* isolates (Fig. 1). In the isolate P516a, plasmid DNA was present at high enough concentration to allow detection by visual inspection of the strains' mtDNAs which had been digested with *EcoRI*, electrophoresed on an agarose gel and stained with ethidium bromide. Southern⁵ hybridization of the DNA from this gel with cloned plasmid monomer (pNc516P, ref. 6) demonstrated that six Louisiana strains and three from other locations also carried homologous plasmid of the same monomer length (Fig. 1b) at a copy number of 10^{-3} to 10^{-4} that in P516a. When the occurrence of the plasmid is compared to the geographic locations and to the mitochondrial types² with which it is associated, the following observations suggest that independent loss or gain of this molecule is occurring: (1) there is no one-to-one correspondence of mtDNA type² or location with the presence or absence of the plasmid, and (2) strains well outside Louisiana and with divergent mtDNA types² may carry plasmid molecules.

To account for the distribution of mitochondrial plasmid DNAs, we examined the possible loss of plasmid during asexual spore production (conidiation). We searched for plasmid in 100 strains, each having arisen from a single conidium of the plasmid-bearing isolate, P516a. Every conidial strain had plasmid as judged from hybridization of pNc516P to dot blots of miniprep total DNA⁷ by the method described in Fig. 2.

We then examined plasmid gain or loss during sexual reproduction. We analysed progeny of reciprocal matings between the plasmid-bearing (P+) isolate P516a and the plasmid-free (P-) isolate 3960A for mtDNA type and plasmid. The mtDNA of each parent could be distinguished because P516a mtDNA has two insertions relative to 3960A mtDNA (Fig. 1).

We collected 130 mature ascospore progeny from matings of P+ males and P- females, and 100 mature ascospore progeny from the reciprocal mating. Dot blot hybridizations between total progeny DNA and pNc516P showed eight progeny of P- females with plasmid and 14 progeny of P+ females without plasmid. These putative P+ progeny of P- females, and putative P- progeny of P+ females were examined further by *EcoRI* digestion and electrophoresis of total DNA⁷, followed by Southern hybridization with pNc516P to search for plasmid DNA and hybridization with *N. Crassa* 74-OR23-1A mtDNA to determine the mtDNA type. All 14 putative P- progeny of P+ females contained plasmid DNA at very low copy number and therefore were not P-.

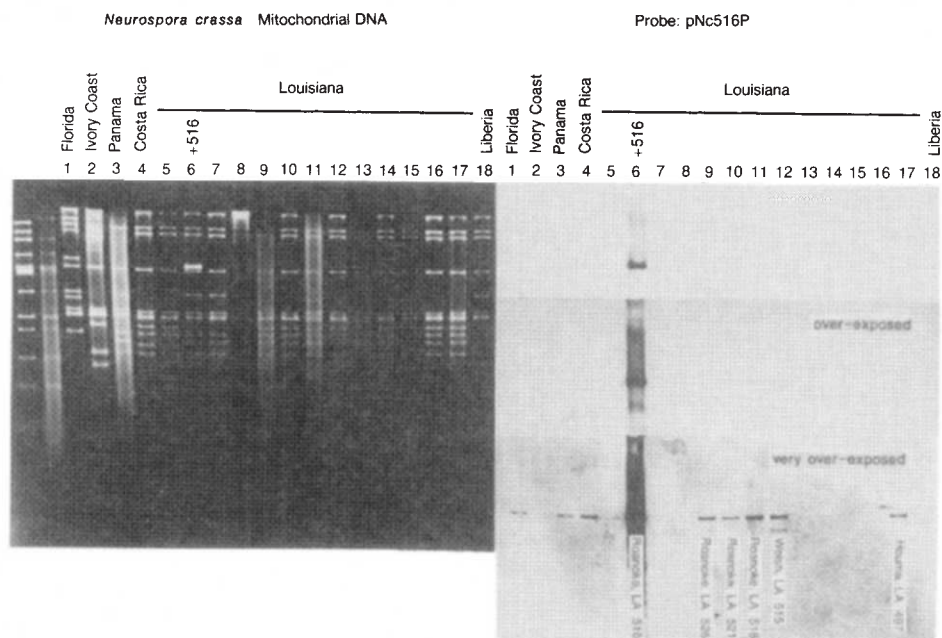
All of the eight putative P+ progeny of P- females had 5.2 kilobase-pair (kbp) bands homologous to pNc516P and were truly P+. All progeny carried maternal mtDNA and the paternal mtDNA patterns were not detected. Three of the P+ progeny, X26, X40 and X44, had the plasmid at copy numbers approximately 10^{-3} of that in P516 (Fig. 3). Two of these strains, X26 and X40, lost the plasmid during subculturing (for example, X26, Fig. 3), whereas the remaining strain, X44, maintained its plasmid population. We detected plasmid in DNA isolated from purified mitochondria and purified nuclei of X44 by Southern hybridization to pNc516P (Fig. 4). But densitometry of autoradiographs from Southern hybridizations between the nuclear and mitochondrial DNAs and both cloned plasmid⁶ and cloned *N. crassa* mtDNA fragments⁸ showed that all plasmid in the nuclear DNA preparations could be accounted for by mitochondrial DNA contamination (see Fig. 4 legend). We conclude that plasmid DNA detected in X44 mitochondrial preparations represents plasmid inherited from the paternal parent which entered the maternal mitochondrial population.

Our results confirm maternal inheritance of mtDNA in *N. crassa*^{3,4} even when the plasmid molecule is paternally inherited. Previous studies of mitochondrial plasmid DNA inheritance analysed relatively few progeny and analyses did not include DNA/DNA hybridization^{9–11}. The mechanism that prevents

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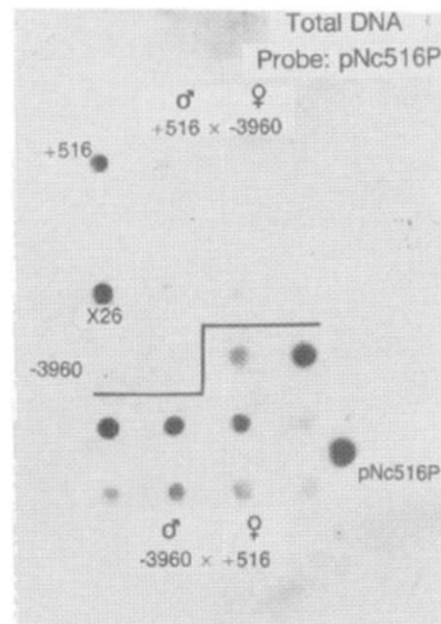
FIG. 1 Detection of mitochondrial plasmid DNAs in mtDNAs of natural *N. crassa* isolates by hybridization to plasmid DNA cloned from *N. crassa* P516 (plasmid: pNc516P, ref. 6). *a*, Agarose gel of *N. crassa* mtDNAs digested with *Eco*RI. Lanes: 1, Groveland, FL FGSC 1945; 2, Adiopodoumé, Ivory Coast FGSC 430; 3, Canal Zone, Panama FGSC 1131; 4, Coto, Costa Rica FGSC 851; 5, Houma, LA P501; 6, Roanoke, LA P516; 7, Welsh, LA P507; 8, Houma, LA P491; 9, Roanoke, LA P526; 10, Roanoke, LA P521; 11, Roanoke, LA P518; 12, Welsh, LA P515; 13, Welsh, LA P512; 14, Welsh, LA P509; 15, Welsh, LA P506; 16, Houma, LA P503; 17, Houma, LA P497; 18, Liberia FGSC 961. Note the high copy number plasmid DNA in lane 6. An additional experiment, not included here, demonstrated the presence of plasmid in Roanoke, LA P524. Note that P516a mtDNA has two insertions relative to lane 18 (Liberia) mtDNA, one of 80 bp in *Eco*RI fragment 9 and another of 50 bp in *Eco*RI fragment 5 (incorrectly placed in *Eco*RI-3 in ref. 2; Taylor and Natvig personal communication). 3960A mtDNA appears identical to mtDNAs of laboratory wild-type 74-OR23-1A and Liberia 961. *b*, Increasingly longer development of Southern⁵ hybridization between DNAs in *a* and biotinylated pNc516P. The amount of plasmid detected in lane 1 represents 10⁻⁴ of that present in lane 6.



METHODS. Isolates were obtained from the Fungal Genetics Stock Center (FGSC) or were a gift from David Perkins, Standord University (see ref. 21). DNAs were isolated from purified mitochondria^{22,23}, digested, electrophoresed, blotted to nylon and hybridized to biotinylated pNc516P, a recombinant plasmid containing the entire mitochondrial plasmid from P516 (ref. 6). Hybridized pNc516P was detected by conjugation with and localization of alkaline phosphatase-avidin complexes as described by the supplier (ENZO, Biorad; ref. 6).

FIG. 2 Screening for plasmid DNA in progeny of P+ male × P- female matings (above the line) and P- male × P+ female matings (below the line). Total DNA was isolated from single ascospore colonies, blotted on nylon and hybridized to pNc516P. Control DNAs were prepared in the same way from the P+ (516) and P- (3960) parents and were blotted along with purified pNc516P. No P- progeny from the P- male × P+ female matings are seen in this figure. In total, 8 putative P+ progeny were found by screening 130 progeny of the P+ male × P- female matings and 14 putative P- progeny were found by screening 100 progeny of P- male × P+ female matings.

METHODS. Matings between the P+ parent (P516a, natural isolate from Roanoke, LA) and the P- parent (3960A, *al-3*, *per-1*; compare with ref. 24) were made on synthetic crossing medium²⁵ with 1.5% agar and 2.0% sucrose as described previously²⁶. 3960A has white ascocarps and because this is under control of the female parent (see ref. 24), female parentage could be confirmed. The female strain was inoculated and allowed to grow and produce protoperithecia for three days at 30 °C in darkness followed by two days at 22 °C with 12 h light per day before being fertilized with conidia or hyphal segments from the male parent. Mated cultures were incubated under the same conditions until perithecia matured, ~14 days. Any perithecia developing from germinated male elements were removed before perithecia matured. Ascospores were recovered after discharge from the Petri dish lid or from mature perithecia by homogenization in sterile water with 0.1% Tween 80, induced to germinate by heat shock at 60 °C for 90 min, and plated at low density on Petri plates of N agar with 2.0% sucrose²⁷. Individual germinated ascospores were transferred to fresh N agar plates, incubated at 30 °C until mycelium covered the plate and stored at -20 °C. Total DNA was prepared by mini-prep⁷ from subcultures of progeny colonies grown in liquid N medium²⁷, frozen in liquid nitrogen, lyophilized, and ground dry at room temperature. Total DNA was spotted on nylon membrane with the aid of vacuum and hybridized to radiolabelled pNc516P as described previously^{6,8}.



paternal transmission of mtDNA during sexual reproduction is unknown, but plasmid molecules must either be able to move through the trichogyne into the ascogonium independently of their host mitochondrion or be carried by male nuclei. Although we detected plasmid sequences in nuclear preparations of P516a, our control experiments show that contamination by mitochondria can account for this plasmid DNA. Stable inheritance of infective plasmids may further require their incorporation into

the mitochondria of the female and then the progeny, because the one strain that maintained plasmid during subculturing, X44, harboured plasmid in the mitochondria.

Horizontal gene transfer, and not extremely slow evolution, has been invoked to explain the presence of highly conserved nuclear genes or DNA sequences in otherwise strongly diverged organisms¹²⁻¹⁵. Our data may reflect a special case of horizontal transfer where the plasmid has moved between otherwise

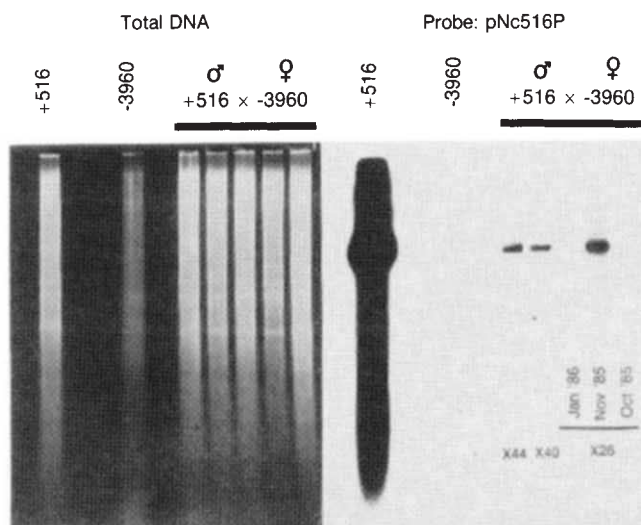


FIG. 3 Detection by Southern^b hybridization of plasmid DNA in total DNA of P+ male $\times$ P- female matings. *a*, Agarose gel of *Eco*RI-digested total DNA from the P+ male parent (P516), the P- female parent (3960) and three P+ progeny (X44, X40 and three subcultures of X26 made at three different times from the original ascospore isolation plate: October 1985, November 1985 and January 1986). *b*, Hybridization between the DNAs shown in *a* and radiolabelled pNc516P. In overexposed autoradiographs DNA homologous to pNc516P is present in each P+ progeny. Plasmid DNA was not detected in every subculture of X26. In other experiments, DNA homologous to pNc516P was detected in the remaining five P+ progeny at lower copy number than the progeny shown here. All P- progeny from the P- male $\times$ P+ female matings were found to contain plasmid DNA at low copy number.

METHODS. Mating, culturing and total DNA extraction were as described in Fig. 2. Restriction enzyme digestion, nylon blotting and hybridization to radiolabelled plasmid were as described in Fig. 1.

isolated mitochondrial DNA lineages. The possible evolutionary relationship of fungal mitochondrial introns and plasmids is intriguing and horizontal transfer in a manner reminiscent of mammalian retroviruses¹⁶ has been implicated for these molecules^{17,18}. A similar case is found in *Brassica* species where mitochondrial plasmids are conservative compared to the crucifer's plastid and mitochondrial genomes and phylogenetic analysis supports their independent transfer¹⁹. Mitochondrial plasmid transfer has been demonstrated in protoplast fusion experiments for *Brassica*²⁰.

Previously, we had proposed that *Neurospora* plasmids were either present before species divergence and were evolutionarily conservative, or that plasmids were horizontally transferred¹. Because *Neurospora* mtDNA and plasmids differ in their mode of evolution, primarily by length mutations in mtDNA² and by nucleotide substitution in plasmids⁶, direct evolutionary comparison was inappropriate. Within *N. crassa*, horizontal transfer of these plasmids is now supported by two lines of evidence presented here, the distribution of plasmid DNAs in natural populations and the experimental transfer of plasmid independent of the host mitochondrion at an evolutionarily significant rate. □

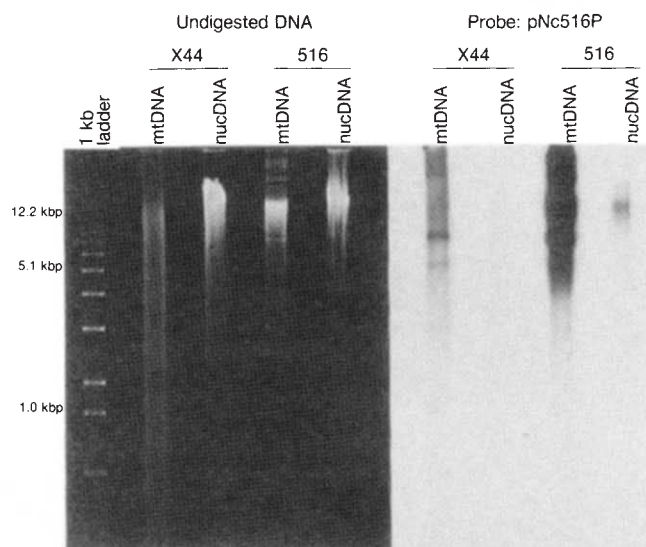


FIG. 4 Detection of plasmid DNA in mitochondria of X44. *a*, Undigested mitochondrial and nuclear DNAs of P516 and progeny X44. *b*, Southern hybridization of DNAs in *a* and radiolabelled pNc516P. Lanes as in *a*. The amount of plasmid DNA detected in the mitochondria of X44 is approximately 17% of that in the mitochondria of P516, relative to the amount of mitochondrial DNA. Small amounts of plasmid DNA detected in both P516 and X44 nuclear preparations can be accounted for by contamination of nuclear preparations by mitochondrial DNA.

METHODS. Mitochondrial DNA of X44 and P516 isolated as in Fig. 1. Nuclear DNA isolated according to the method of Orbach *et al.*²⁸. Mycelial cultures as in Fig. 1 and split for mitochondrial and nuclear analyses. The blot shown in *b* was probed with both cloned plasmid (pNc516P; ref. 6) and cloned *N. crassa* mtDNA fragments (pUCB7a and pUCB7b; ref. 8) and densitometry was used to measure the signal strengths on the autoradiographs. We expected that if plasmid DNA was present in nuclei (independent of mtDNA) and there was some contamination of nuclear preparations by mitochondria, then the ratio of plasmid DNA to mtDNA for nuclear preparations should be greater than or equal to that ratio for mitochondrial preparations. The ratio of plasmid DNA to mtDNA was less in the nuclear preparations than in the mitochondrial preparations in both X44 and the P+ parent, P516a (plasmid DNA signal/mtDNA signal: X44 nuclear = 0.11, X44 mitochondrial = 0.29, P516a nuclear = 0.88, P516a mitochondrial = 1.62). We calculated that the level of plasmid in X44 is approximately 17% of that in P516 by using the ratios of plasmid DNA signal/mtDNA signal for the mitochondrial preparations of the two strains ($0.29/1.62 \times 100\% = 17\%$). Probing with *N. crassa* ribosomal DNA (pMF2; ref. 29) showed no nuclear contamination of mtDNA preparations.

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